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CLINICAL AND EXPERIMENTAL

PRODUCTION OF PYROGEN BY SOME BACTERIA*

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HISTORICAL REVIEW

IN 1911 Hort and Penfold showed that the intravenous injection of freshly distilled water caused no symptoms in animals and in men, while the intravenous injection of the same water, after it had been incubated in unsterile vessels, caused a febrile reaction. Since the fever-producing principle was still present after the water had been passed through a Berkefeld filter, these authors postulated that this principle, provisionally called pyrogen, was a product associated with bacteria but was not a part of the bacterial bodies themselves.

In 1923 Siebert, studying the febrile reaction following the intravenous administration of distilled water in rabbits, confirmed the findings of Hort and Penfold, and showed conclusively the bacterial origin of pyrogen. With Bourn, Siebert in 1925 studied 25 strains of bacteria isolated from distilled waters and reported that those responsible for the production of mild fevers were related to the Jordan group X of river-water bacteria, those producing severe fevers to group XI, while the nonpyrogenic bacteria fell into group XIII, which were chromogenic. According to a more recent classification (Bergey, 1930), these organisms would all be in the family Bacteriaceae, with the strong and mild pyrogen producers belonging to the genera Achromobacter and Escherichia, and the nonpyrogenic organisms to several genera of the tribe Chromobacterieae.

Banks in 1934 reported that *Pseudomonas ureae* and *scissa*, two species of the genus Pseudomonas which he isolated from surface waters, were pyrogenic. Apparently not familiar with the more extensive bacteriologic work of Bourn and Siebert, he stated categorically that these two organisms were specific pro-

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ducers of pyrogen, and that pathogens, as represented by *Staphylococcus aureus*, and nonpathogens, as represented by *Bacillus subtilis*, were productive of a delayed febrile response and, therefore, could not be classified as pyrogen producers. Banks's work, while containing many conclusions now found untenable, added one more genus to those found by Bourn and Siebert as pyrogen producers and refuted Siebert's impression that pyrogenicity was associated with achromogenicity.

Thus far the study of pyrogen production has been confined to the bacteria found in waters. However, the striking parallelism between the symptomatology of a pyrogenic reaction from "reactive" waters, and that caused by the intravenous injection of typhoid vaccine, or of acacia and sera made "reactive" by the implantation of other strains of bacteria, indicates the close kinship of these fever-producing principles. This kinship is made still closer by the similarity of their filtration characteristics as shown by Co Tui, Benaglia, Ruggiero, and Yates.

It thus appears that pyrogen production may be much more widespread than was first embraced in the concepts of Siebert and Banks. The present work is an attempt to determine how universal this phenomenon is.

EXPERIMENTAL PROCEDURE

Eighteen strains of microorganisms belonging to different genera, tribes, and families were investigated. They were prepared for tests in three different ways.

In the first series 12 bacterial strains grown in initially pyrogen-free broths were used. The cultures were incubated for forty-eight hours at their particular optimum temperatures, and then centrifuged at 4,000 r.p.m. for forty-five minutes in an angle centrifuge. The supernatant fluid was filtered through a Berkefeld "W" filter, and the sterile filtrate was tested. These tests may be considered primary tests, since the immediate media in which the organisms grew were used.

The second series consisted of bacteria which were more convenient to grow on slants or in Kolle flasks. The colonies were washed out with a few cubic centimeters of pyrogen-free physiologic saline solution containing 0.4 per cent phenol, inactivated for forty-five minutes by heat. The suspension was filtered as in the case of broth cultures, and the filtrate was tested. These tests may likewise be considered primary tests, since whatever pyrogen was present in the colonies would presumably go into solution in the suspending media. Four organisms were tested in this way.

The third series consisted of nine organisms tested in the form of vaccine made with pyrogen-free water. The first three were highly pathogenic organisms; namely, *Vibrio comma*, *Neisseria intracellularis*, and *Pasteurella pestis*. These organisms were prepared in sets of two; one set by a biological firm and the other by the bacteriologic department of the university. The other six organisms in this series had been tested in series one, so that the tests in this case serve merely as a check. All these vaccines were passed through a Berkefeld filter, as in the case of the other two series and the cell-free filtrate used. The

tests in this series may be thought of as secondary tests, since the media tested were not in immediate contact with the living bacteria. While a positive finding may be considered significant, a negative finding may only mean that the pyrogen produced may be so slight as to be lost in the process of making the vaccines.

A positive pyrogenic reaction in the dog, as reported in our previous communications, consists of a leucopenia thirty to forty-five minutes after the intravenous injection of the pyrogenic material; a rise of temperature which begins to be perceptible thirty minutes after injection, reaching a peak in from two to four hours and returning to normal in four or five hours. In mildly severe reactions there are prostration and gastrointestinal symptoms, such as vomiting and diarrhea, both of which may contain blood. These symptoms are comparable to those in men.

The dog is used throughout this work not only because of the consistency of its temperature curve, but also because the constancy of the leucopenia and the frequent appearance of the secondary symptoms of prostration and gastrointestinal disturbances serve as an added check.

That this pyrogenic reaction is not due to nucleic acid is shown in three experiments in which the intravenous injection of 1, 3, and 5 mg. of pure nucleic acid, prepared by Dr. P. A. Levene, into each of three dogs failed to provoke a similar response.

DISCUSSION OF RESULTS

Table I summarizes the results of this investigation. The first four organisms have not been classified as to either species or genera, but belong to the family of the Bacteriaceae of the tribes Achromobactericae and Chromobactericae. W14B and W14C were isolated from well water, and 31y and 2WS from pond water. Their best growth was obtained at 20° C. They were gram-negative bacilli, varying in shape from minute bacillary forms in the case of W14B to a large coccoid form in the case of W14C. 31y was a small bacillus exhibiting chromogenic properties and imparting to the broth and agar preparations a light yellow color. 2WS was the largest bacillus of the group and often grew in long chains.

All these four organisms were included here to show the similarity of the pyrogenic reaction elicited by the injection of their products and that elicited by the injection of the products of microorganisms of other groups.

Each item listed in Table I has been selected from a series of at least five experiments as the one best representative of that series.

A study of the table shows a number of interesting facts. The first 13 organisms listed in Table I are all pyrogen producers, 9 of them not being members of the river-water group. Four of these, namely, *E. typhi*, *Streptococcus pyogenes*, *Vibrio comma*, and *N. intracellularis* are pathogens. Two others, *E. coli* and *Staphylococcus aureus*, are facultative saprophytes. It will also be seen that it takes different amounts of the broth filtrate to produce a reaction of an intensity similar to the range of reactions common to the pyrogen producers in the table, the dose ranging from 1 c.c. to 15 c.c. While this may be interpreted to mean that some microorganisms are better pyrogen producers

TABLE I

ORGANISM	FILTRATE TESTED	AMOUNT USED (C.C.)	CONC. PER C.C. VACCINE IN BILLIONS	DOG WEIGHT (KG.)	W.B.C. DROP (THOUSANDS)	CHANGES IN TEMP. (° F.)	SYMPTOMS
W14B	B	10		16	14.5-4.3	101-104	Shivering, emesis and bloody stool
	V	5	20	9	19.6-3.0	102.4-104.8	None
W14C	B	10		16	12.2-3.2	100.2-103.2	Violent shivering, emesis, and bloody stool
	V	1	10	9	22.9-4.6	102-105	Violent shivering
31y	B	5		11	17.6-4.7	101.6-103.6	Violent shivering and emesis
	V	5	20	11	15.2-2.2	101.6-103.6	Shivering
2WS	B	5		17	15.5-4.2	101.6-106.4	Shivering, emesis, and defecation
	V	15	20	9	15.4-5.2	101.4-104.6	Shivering and emesis
Escherichia coli	B	1		11	15.5-2.1	101.6-103.6	Violent shivering and emesis
	V	1	10	13	21.1-4.6	101.4-103.6	Violent shivering
Eberthella typhi	B	1		11	11.9-2.9	102-104	Shivering, emesis, and defecation
	V	1	10	11	20.7-4.1	101.8-104.6	Shivering, emesis, and defecation
Streptococcus pyogenes	B	7		8	14.8-7.6	101.8-103.6	Shivering, emesis, and bloody stool
Proteus vulgaris	B	1		13.5	11.9-2.9	102-104	Shivering, emesis, and defecation
Staphylococcus aureus	B	10		13	38.6-25.0	102.8-104	Shivering, emesis, and defecation
Vibrio comma	V	1	3	9	16.9-4.3	101.8-106	Emesis, shivering and defecation
Neisseria intracellularis	V	8	1	16	3.7-0.8	101.4-104.6	Emesis and defecation
Saccharomyces Pink wild yeast	B	15		16	22.5-6.9	100.6-103.0	None

B – Broth.
V – Vaccine.
S – Suspension.
Toxin broths heat inactivated.

All sterile broth controls used in culturing organisms gave no reactions on intravenous injections in dogs, i.e., no changes in temperature, white blood cell count, or any systemic symptoms.

TABLE I—CONT'D

ORGANISM	FILTRATE TESTED	AMOUNT USED (C.C.)	CONC. PER C.C. VACCINE IN BILLIONS	DOG WEIGHT (KG.)	W.B.C. DROP (THOUSANDS)	CHANGES IN TEMP. (° F.)	SYMPTOMS
Bacillus subtilis	B	5		16	34.0-3.5	102.8-105.6	Defecation with blood, animal depressed
Corynebacterium diphtheriae	B	17		21	10.2-9.5	101.2-100.8	None
	S	12		12	16.3-16.4	101.6-101.4	None
Pasteurella pestis	V	5	5	9	16.6-13.5	100.8-100.8	None
Pasteurella cuniculicida	S	12		10	24.5-20.1	101.6-101.6	None
Mycobacterium tuberculosis	S	15		18	36.0-36.0	102.6-102.8	None
Neisseria gonorrhoeae	S	17		12	17.9-10.0	102.0-102.0	None

than others, yet it could also mean that the rates of rapidity of their proliferation may be different. Unless bacterial counts are made of the cultures, this point cannot be settled.

In the case of the vaccines, however, where some quantitation has been used, the indication that some bacteria are better pyrogen producers than others is stronger. Thus of the 8 organisms tested in the vaccine form, it takes only three billion cells of *V. comma* to produce a cell-free vaccine filtrate which causes a temperature rise of 4.2° F., ten billion in the case of *E. coli* to produce a rise of 3.1° F., and ten billion of *E. typhi* to produce a rise of 2.8° F., while it takes 300 billion of 2WS to produce a rise of 3.5° F. These figures would seem to indicate that *V. comma* is the most efficient producer of pyrogen among these strains with *E. coli, E. typhi, N. intracellularis,* and W14C second and 2WS the least.

Of practical importance from the viewpoint of air contamination in the production of pyrogen in the preparation of medicaments and other chemical solutions is that *Proteus vulgaris* and *B. subtilis* are pyrogen producers.

It is also of particular significance that wild yeast produces pyrogen. This opens the field for further experimentation in group of yeast and molds.

The last five microorganisms in the table; namely, *C. diphtheriae, Pasteurella pestis, Pasteurella cuniculicida, M. tuberculosis* and the *gonorrhoeae* were according to these tests nonpyrogenic. All these were subjected to primary tests, except *Pasteurella pestis,* which was tested only in the vaccine form, using an amount equivalent to 25 billion cells. *Pasteurella pestis* is thus at best a weak pyrogen producer if at all. In all these negative tests it is interesting to note

TABLE II

CLASS SCHIZOMYCETES

ORDER EUBACTERIALES

FAMILY	TRIBE	GENUS	ORGANISM	PYROGEN PRODUCTION	INVESTIGATOR
Coccaceae	Streptococceae	Streptococcus	pyogenes	Positive	
	Neisserieae	Neisseria	gonorrhoeae	Doubtful	
	Neisserieae	Neisseria	intracellularis	Positive	
	Micrococceae	Staphylococcus	aureus	Positive	Co Tui, Schrift, and Banks
Spirillaceae		Vibrio	comma	Positive	
Bacteriaceae	Chromobacterieae	Serratia	ruber	Negative	Bourn and Siebert
	Chromobacterieae	Flavobacterium	radiatum	Negative	Bourn and Siebert
	Chromobacterieae	Flavobacterium	ochraceum	Negative	Bourn and Siebert
	Chromobacterieae	Flavobacterium	arborescens	Negative	Bourn and Siebert
	Chromobacterieae	Pseudomonas	urea	Positive	Banks
	Chromobacterieae	Pseudomonas	scissa	Positive	Banks
	Chromobacterieae		31y	Positive	
	Achromobacterieae	Achromobacter	pinnatum	Positive	Bourn and Siebert
	Achromobacterieae	Achromobacter	solitarium	Positive	Bourn and Siebert
	Achromobacterieae	Achromobacter	candicans	Positive	Bourn and Siebert
	Achromobacterieae	Achromobacter	refractans	Positive	Bourn and Siebert
	Achromobacterieae	Achromobacter	tiogense	Positive	Bourn and Siebert
	Achromobacterieae	Achromobacter	punctatum	Positive	Bourn and Siebert
	Achromobacterieae	Achromobacter	lacticum	Positive	Bourn and Siebert
	Achromobacterieae	Achromobacter	W14B	Positive	
	Achromobacterieae	Achromobacter	W14C	Positive	
	Achromobacterieae	Achromobacter	2WS	Positive	
	Bacterieae	Escherichia	formica	Positive	Bourn and Siebert
	Bacterieae	Escherichia	coli	Positive	
	Bacterieae	Proteus	vulgaris	Positive	
	Bacterieae	Eberthella	typhi	Positive	
	Pasteurelleae	Pasteurella	pestis	Negative	
	Pasteurelleae	Pasteurella	cuniculicida	Negative	
Bacillaceae		Bacillus	subtilis	Positive	Co Tui, Schrift, and Banks
		ORDER ACTINOMYCETALES			
Mycobacteriaceae		Mycobacterium	tuberculosis	Negative	
		Corynebacterium	diphtheriae	Negative	

that the white blood cell count did not undergo any substantial change except in the case of the *N. gonorrhoeae* test in which there was a suggestive fall.

It is clear from these studies that the matter of pyrogen production is not associated with pathogenicity as first postulated by Banks or with chromogenicity as postulated by Siebert (1923), or with the property of taking or rejecting the Gram stain as suggested by Cecil.

In Table II are compiled all the bacteria tested for pyrogen production from data furnished in the work of Siebert, of Banks, and in our present work. It will be seen that the greatest number tested have been selected from the order of the Eubacteriales of the class Schizomycetes, with particular emphasis on the family Bacteriaceae. In addition to the three tribes of the family Bacteriaceae that Siebert found pyrogenic and to the one genus of the tribe Chromobacterieae by Banks, the present work has added four families of the order Eubacteriales, one family of the order Actinomycetales, embracing a total of eleven known genera and thirteen known species.

SUMMARY

In a study of pyrogen production by representatives of different orders, families, genera, and species of bacteria, it was found that this phenomenon is a more widespread one than has hitherto been envisaged. It is not confined to any one group, but occurs in a number of different groups. Nor is it associated with nucleic acid, with pathogenicity, chromogenicity, nor with the property of taking the Gram stain. The production of pyrogen by a member of the yeast group suggests that even higher forms than bacteria may possess this property of pyrogen production. How pyrogen is related to the various toxic substances isolated by various workers from different bacteria remains to be studied. This statement implies that chemically and immunologically pyrogen is still an unknown entity. It is interesting to note that in this work a physiologic criterion has been used for the study of a bacterial product, a criterion distinct from those in use heretofore, namely, death or the production of antibodies in experimental animals. It is quite conceivable that there may be some bacterial products which are neither lethal nor antigenic and are, nevertheless, physiologically potent.

We wish to acknowledge our indebtedness for the aid in the preparation of experimental material given by Dr. Julius A. Klosterman, of the Department of Bacteriology, New York University, College of Medicine, by the Baxter Laboratories of Glenview, Ill., and the Sharp & Dohme Company, Philadelphia, Pa. To the late Dr. P. A. Levene we are indebted for the preparation of pure nucleic acid.

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INTESTINAL VASCULAR SCLEROSIS*

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INTESTINAL vascular sclerosis is a condition of the mesenteric blood vessels usually associated with old age and often accompanied by disordered bowel function.

Pathology.—Narrowing of the extramural and intramural mesenteric vessels is part of a more generalized systemic condition of degenerative vascular disease associated with senescence. From the pathologic viewpoint these changes consist chiefly of disruption or disappearance of the elastic lamina, fatty degeneration with subintimal proliferation, calcific deposition, and atrophy of the media. The end result is a narrowed, rigid vessel, which is unresponsive to the ever-changing circulatory requirements of the organs supplied. The most advanced disease is generally seen in the larger arteries, where actual widening of the lumen may occur. This is essentially due to weakening of the wall from degeneration of the media and elastica. In the smaller arteries, however, such as those seen in the intestinal submucosa, the most common change noted is a subintimal proliferation of connective tissue. This deposition of connective tissue is often accompanied by thickening of the elastica, both representing an adaptive overgrowth to bolster the overlying weakened media. Occasionally this process occurs in young people following systemic infections, but its prevalence in the older age groups appears to be associated with the biologic process of aging. Complete obliteration of the intramural intestinal vessels sometimes occurs and can be readily detected at necropsy by subjecting the opened bowel to transillumination.[1] They stand out as straight, tapering, white, bloodless, threadlike filaments. By careful section of selected areas one can often trace successive stages in the same vessel. In some instances narrow clefts form in the fibrous tissue which obliterates the vessel lumen. These clefts are lined by endothelial cells; they often contain erythrocytes and represent an attempt at recanalization. In this manner it appears quite possible that a fair blood supply may be maintained, though it is probably inadequate as compared with that carried by the normally patent vessel.

Clinical Aspects.—In mild, moderate, or recanalized forms of intestinal vascular sclerosis few or no symptoms may be present. In patients with the more advanced type of disease the chief clinical symptoms and signs are abdominal distention, constipation, indigestion, and abdominal cramps after eating. One or all may be present, depending upon the degree of vascular narrowing. They are most frequently encountered in the sixth and later decades of life, but they may be present earlier. The adage "a man is as old as his

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arteries'' is applicable to the intestinal vessels. ''Senescence'' is a relative term, however, as Boas[2] has recently pointed out, varying considerably in different persons. Thus, relatively young people may have intestinal vascular sclerosis, and some old people are comparatively free of it (Humphrey,[3] Groddeck[4]). The former generally exhibit concomitant changes in the glomerular tufts and larger renal vessels with persistent hypertension, either because of a glomerular nephritis complicating some infectious disease or as the late phase of essential hypertension with arteriolosclerosis. In older people, however, hypertension is not invariably present any more than in coronary sclerosis, or sclerosis of the cerebral arteries. Intestinal vascular sclerosis is essentially a focal manifestation of generalized arteriosclerosis. The thought has often occurred to us that the epigastric and other abdominal discomfort in coronary disease is often due to mesenteric sclerosis. The symptoms of which patients with mesenteric sclerosis complain are often regarded as being on a purely functional basis. This, however, is not the case, as can be shown by careful general physical examination and sigmoidoscopy. The changes noted by the latter method are distinctive and readily elicited by examination of the submucosal intestinal vessels. During life, with blood coursing through the vascular arterial branches, their relative thickness, elasticity, and general contour can be determined with the aid of a telescopic device and green color filter[5] (Wrattan X). Normally, the submucosal vessels can be seen as graceful, delicate, long, red, sinuous undulations, gradually tapering to their tips. Their elasticity can be determined to some extent by means of intermittent suction applied through the inflation tube of the sigmoidoscope. In this manner a transient period of negative pressure is created. Normal vessels, alternately redden and pale, correspond to the phases of negative and positive pressure. With advancing sclerosis and narrowing of the lumen the arteries become thicker, the undulations abrupt, tortuous, or corkscrew, and alternate suction and release produce no appreciable reddening and paling of the vessels. During the late or obliterative stage the vessels pursue a short straight course, terminating rather abruptly in white bloodless cords which do not respond to suction. Examination of the eye grounds is suggested as a supplementary procedure to sigmoidoscopy, for the retinal vessels parallel the intramural intestinal branches both as to size and extent of pathology.

The mechanism underlying the symptomatology in intestinal vascular sclerosis appears to be a transient or permanent ischemia with relative anoxemia. The bowel is a hollow muscular organ, with an exceedingly rich blood supply that must be adequately maintained for effective contractility and functioning of the digestive glands. Interference with the blood and oxygen requirements of the musculature produces spasm (i.e., muscle cramp) and later, atony. Barker[6] states that in case histories of 300 patients over 60 years of age, digestive symptoms were second highest in frequency. While many factors must be considered, such as inadequate mastication, there still remains a large group of persons whose symptomatology can only be explained by some factor incident to the aging process. Patients will often state that small amounts of easily digested food are better tolerated than large meals, an ob-

servation that many of our keen predecessors put to practical use in the treatment of elderly patients with digestive complaints. The reason is obvious, since easily digested foods in small amounts require less work on the part of the intestine for their assimilation than bulky or indigestible foods. The impaired blood supply under these circumstances may be adequate because the digestive requirements have been adapted to the functional capacity of the bowel.

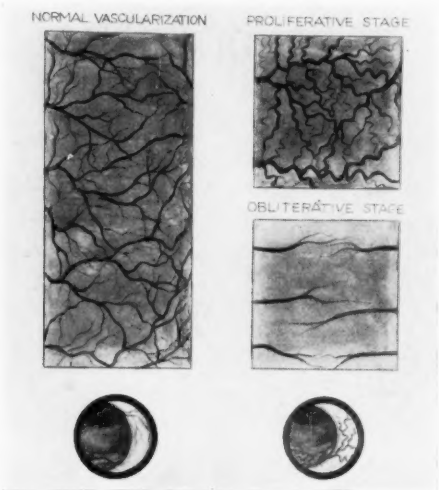

Fig. 1.—Arteriosclerosis. Mesenteric vessels thickened tortuous, or partially obliterated. Corresponds in individual cases to appearance of retinal vessels.

With advancing years, as the vascular changes progress, the intestinal musculature tends to lose its tone. This is particularly true in the colon where gas, which is normally present, is not passed readily and tends to accumulate with increasing atony of the wall, thus perpetuating a vicious cycle. The chief signs of intestinal atony are distention and constipation. In this connection it may be of interest to recall the relationship of distention to impaired gas diffusion through the intestinal wall. Normally, as pointed out by McIver, Redfield, Benedict,[7] and others, the intestinal wall serves as a semipermeable membrane, permitting diffusion of gases in either direction, i.e., from the intestinal lumen to the blood, or vice versa. Any interference in the free passage of gases may lead to flatulence (Dunn and Thompson).[8] Alvarez,[9] Kato,[10] Kader,[11] Schoen,[12] and others regard impaired circulation in the mesenteric vessels as one of the prime factors in the production of distention. Schoen stresses, in addition, the lowering in tone of the intestinal muscularis. It is my impression that both factors are closely related to intestinal vascular sclerosis and are often the result of it. In rare instances extreme sclerosis of the mesenteric vessels may result in rupture, i.e., abdominal apoplexy. This subject has been recently reviewed by Crile and Newell,[13] who report ten cases from the literature in addition to their own. Sometimes complete occlusion of a main mesenteric arterial branch occurs with gangrene of the part of the intestine supplied. Here the process usually consists of a superimposed thrombosis in a markedly narrowed lumen.

The frequent occurrence of flatulence in patients with hypertension is well recognized, Kantor and Marks[14] reporting an incidence of over 50 per cent. The association of intestinal vascular sclerosis with long-standing hypertension is, in our experience, quite constant. In reviewing 1,000 necropsies at the Bronx Hospital from the standpoint of concomitant intestinal vascular sclerosis and generalized arteriosclerosis in the same person, agreement was found in all instances where advanced changes were present in the larger vessels. It may be stated that all intestinal necropsy specimens were viewed by means of the transilluminator.

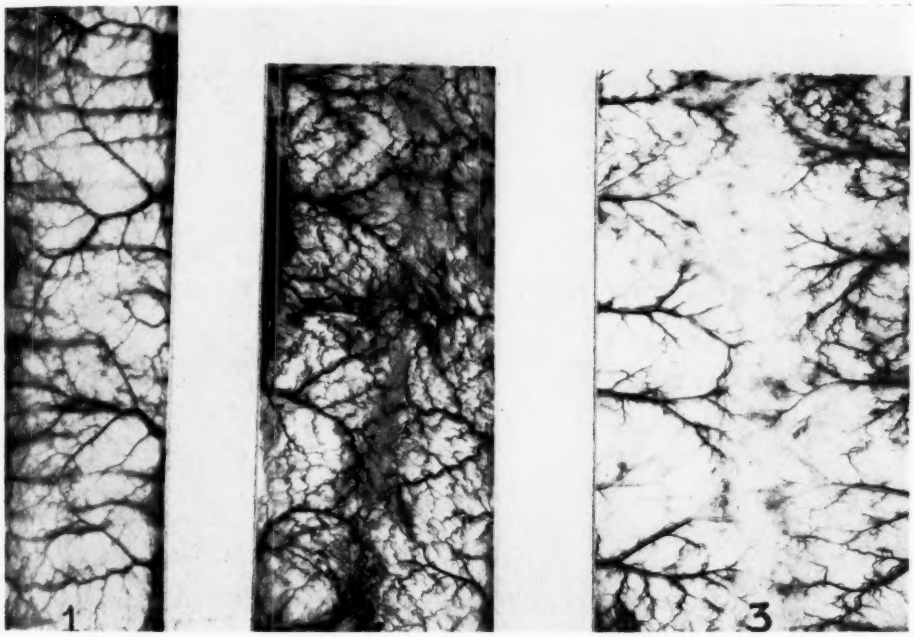

Fig. 2.—Intestinal transparencies. *1*, Normal intramural vascularization. Note long, delicate, sinuous undulations with gradual tapering to finest branches which anastomose freely with adjacent vessels. *2*, Proliferative phase. Vessels are thickened and tortuous. *3*, Obliterative phase. Vessels are straightened and rigid with obliterated terminal arborizations. *2* and *3* are from Case 2 (No. 9048).

Interference with diffusion of gases may be due to a thickened intima or impaired circulation of blood in the intestinal arterioles. In cirrhosis of the liver or failure of the cardiac musculature without arteriolar sclerosis, the fault may be entirely on the venous side.

CASE REPORTS

Group A. Cases illustrating association of intestinal vascular sclerosis and arteriosclerosis elsewhere in the body.

CASE 1.—Hospital No. 61437. Pathology No. 8675. Male, aged 45 years, was admitted on Dec. 5, 1935, with a clinical diagnosis of arteriosclerosis, coronary sclerosis, and coronary thrombosis. The blood pressure was 100 mm. systolic and 80 mm. diastolic. A complete history was unobtainable. Death occurred on the same day. At necropsy the relevant findings were an old thrombosis of the left coronary artery, a recent thrombosis of the right coronary artery, with areas of myomalacia. The entire aorta was the seat of

large atheromatous patches and ulceration extending into the common and external iliac branches. The intramural mesenteric arborizations revealed marked tortuosity and thickening. There was marked coronary sclerosis.

CASE 2.—Hospital No. 59727. Pathology No. 9048. Male, aged 60 years, was admitted on Feb. 13, 1936, and died within a few hours. During a previous admission (Oct., 1935) clinical and electrocardiographic examinations (Dr. Capp) revealed coronary sclerosis with myocardial fibrosis. At necropsy, mural thrombi were present at the apex of the heart and in the right auricle. Both coronary vessels were calcified, and a fresh thrombus was present in the right circumflex branch. An old thrombus occluded the left descending coronary artery 2.5 cm. from its origin. The aorta and other large vessels were markedly sclerotic. There was marked tortuosity and thickening of the intramural vascular arborizations with straightening and obliteration of many terminal branches. There was advanced arteriosclerotic renal disease.

CASE 3.—Hospital No. 74062. Pathology No. 11465. Female, aged 77 years, was admitted on Feb. 8, 1937. The patient was known to have had a mild form of diabetes for fifteen years. Some redness, swelling, and tenderness were present on the little toe of the right foot. There was marked peripheral vascular sclerosis. Oscillometric readings at the ankle and above the popliteal space revealed definite occlusive vascular disease. (Doctors Saland and Klein.) Roentgenographic examination (Dr. Snow) showed marked calcification of the vessels of the extremities. Diabetic gangrene of the right foot and lower third of the leg ensued, and the patient died following mid-thigh amputation on March 22, 1937. At necropsy marked atherosclerosis of the aorta with many calcific plaques and ulceration were noted. The aortic and mitral valve flaps were calcified. The larger arterial branches were similarly involved, particularly the coronary and basilar arteries. Typical arteriosclerotic renal disease was present. The intramural mesenteric branches revealed advanced sclerosis, with tortuous, thickened, and partly obliterated straight vessels.

Group B. Cases with intestinal vascular sclerosis, generalized arteriosclerosis, and marked intestinal clinical manifestations.

CASE 4.—Hospital No. 74413. Pathology No. 11501. Female, aged 56 years, was admitted on Feb. 19, 1937. The chief complaints were progressive constipation, epigastric discomfort, and belching. The patient was known to have had hypertension for two years. Upon admission the blood pressure was 260 mm. systolic and 135 mm. diastolic. The heart was considerably enlarged. Examination of the fundi revealed dilated veins and thin pale arteries, which were highly refractile and sclerotic. Scattered about the posterior aspect were fine yellowish exudates and a few scattered superficial hemorrhages (Dr. Goodfriend). On Feb. 23 roentgenographic examination revealed calcification of the arch of the aorta. There was loss of substance in the tufts of the index, middle, and little fingers on both sides, indicating circulatory deficiency (Dr. Snow). Oscillometric studies (Doctors Saland and Klein) revealed diminished oscillation in the vessels of both ankles, wrists, and right arm. Thermal tests indicated complete absence of reflex vasodilatation in both extremities. These findings pointed to organic obstructive arterial disease. The patient gradually developed uremia with marked nitrogenous retention and died on March 25, 1937. The relevant necropsy findings were a marked ulcerative atherosclerosis of the entire aorta and small granular arteriosclerotic kidneys. The intramural intestinal arterial arborizations were rigid, thickened, and corkscrew-shaped, with partly or completely obliterated straight terminal branches.

The following brief case reports have been selected from a group of 15 to illustrate the possibilities of accurate diagnosis of intestinal vascular sclerosis by sigmoidoscopy and the value of oxygen as a therapeutic agent.

CASE 5.—A. K., female, aged 55 years, complained of recurring attacks of abdominal cramps, constipation, distention, and a sense of epigastric pressure accompanied by labored breathing, particularly at night. Occasionally there was severe precordial pain radiating to both shoulders. Prior to the onset of these symptoms the blood pressure had been 190

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mm. systolic. At the time of consultation it was 150 mm. systolic and 90 mm. diastolic. Cultural, serologic, and parasitologic studies were negative. Sigmoidoscopic and ophthalmoscopic examinations revealed marked sclerosis of the intestinal and retinal vessels. A diagnosis of coronary sclerosis and intestinal vascular sclerosis was made, both being part of a more generalized arteriosclerosis. Considerable relief of abdominal symptoms followed aspiration of the colonic gases and the use of intestinal oxygenation. Several weeks later, after being comparatively free of symptoms, there occurred an acute episode of abdominal pain and an emergency exploratory laparotomy was done at Mt. Sinai Hospital for what appeared to be an acute intestinal obstruction. No obstruction was found, though an omental adhesion in the region of the splenic flexure was present. The striking finding, however, was "a considerable degree of sclerosis in those major abdominal arteries which were seen or felt."[15]

CASE 6.—F. K. G., female, aged 64 years, had recurring attacks of abdominal cramps accompanied by nausea for which she had undergone a cholecystectomy eighteen months previous, but without relief. Physical examination revealed a nervous, apprehensive woman, who exhibited evidence of cerebral arteriosclerosis and mental deterioration associated with senility. There was advanced intestinal and retinal vascular sclerosis. The blood pressure was 140 mm. systolic and 100 mm. diastolic. A diagnosis was made of mesenteric vascular sclerosis with relative anoxemia and intestinal spasm. Temporary abatement of symptoms occurred with intestinal oxygenation, but the mental condition of the patient rendered continued therapy difficult. Roentgenographic studies revealed the presence of a diaphragmatic hernia with diverticulosis (Dr. A. F. R. Andresen). There appeared to be no evidence, however, that the intestinal symptoms were due to either of these conditions. Subsequent studies were carried out at Mt. Sinai Hospital in an attempt to elicit an allergic factor, because of a history of migraine, but these were without success. Several weeks later the patient suddenly died of cerebral apoplexy. It was quite evident in this case that not only were the intestinal vessels sclerotic, but the cerebral, aortic, and peripheral blood vessels were likewise affected.

CASE 7.—B. A., female, aged 58 years, had abdominal cramps, distention, and constipation for two years, which was relieved by rest and quantitative, rather than qualitative, dietary restrictions. Repeated roentgenographic studies were essentially negative. Generalized arteriosclerosis was present with moderately advanced changes in the retinal and intestinal vessels. The condition was relieved by the use of approximately 200 c.c. of oxygen administered by rectum once daily. The patient has increased the total amount of food ingested but still eats rather sparingly by choice. The follow-up is now approximately two years, the patient having completely abandoned the use of enemas to which she was previously addicted. At the present time she has one normal bowel movement daily.

CASE 8.—P. Z., female, aged 70 years, complained of obstinate constipation for many years. For several years the patient noted blood in the bowel movements due to the presence of hemorrhoids. Epigastric and precordial distress with a sense of fullness after meals were present for three years. These symptoms were relieved by rigid restrictions in the quantity of food or by a smooth, low residue diet. There was increasing anorexia and a weight loss of 30 pounds. The essential physical findings were abdominal distention, evidence of hypovitaminosis, and generalized arteriosclerosis. The sigmoidoscopic appearance of the intramural intestinal vascular branches was typical of advanced arteriosclerosis, and similar findings were noted in the retinal vessels. The possibility of coronary sclerosis was entertained, but there was no evidence of an acute thrombosis. With intestinal oxygenation the symptoms were ameliorated with a surprising rapidity that could not be accounted for by psychic or other factors. The appetite improved, all dietary restrictions being removed, and with it the state of vitamin deficiency disappeared. Enemas and other forms of catharsis were immediately stopped, and the patient has had normal daily bowel movements, with rare exceptions, for the past five months. In this case it was felt that the oxygenation made possible the handling of large quantities of food by

the intestinal tract. That the total bulk was not an important factor is attested by the fact that the patient had been constipated before dietary restrictions were imposed by her physician.

THERAPY

The treatment of intestinal vascular sclerosis is based upon a logical consideration of the physiologic and pathologic factors involved. Our present state of knowledge does not point to any agent which can restore to normalcy the narrowed caliber of mesenteric vessels due to the degenerative changes of senescence. Our efforts must be directed toward supportive and corrective measures which will enable a permanently impaired mechanism to handle adequately the limited digestive requirements of old age.

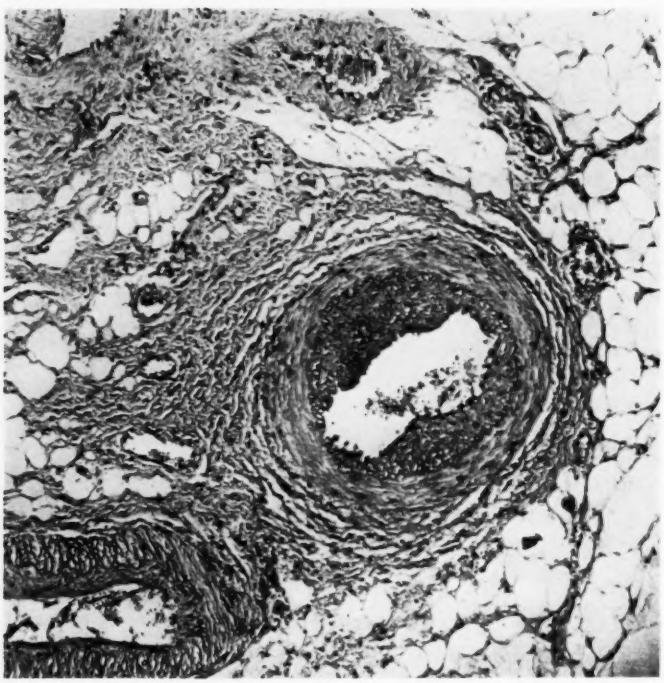

Fig. 3.—Moderate narrowing of the lumen of a mesenteric intramural branch due to subintimal proliferation. Note that elastica is intact.

(a) *General supportive measures.* If signs of myocardial failure or hypertension are present, adequate drug therapy combined with fluid restrictions should be instituted. Supplementary post-prandial rest periods besides the usual rest requirements will prove helpful.

(b) *Dietary limitations.* The caloric requirements of sedentary old age are obviously much less than those of youth or active middle age. Nature appears to be orderly in her plans. With the vascular and other degenerative changes of senescence, there is a lessened tendency to remain physically active, and with it a diminution in the desire for food. Old people become more "picky" in their food habits and are less apt to overeat than was their custom in youth. Violation of this simple rule often brings on abdominal discomfort

so that individuals soon learn their limitations and steadfastly observe them. Simple, easily digested foods, small in total bulk, well masticated and eaten slowly, are indicated. Ordinarily three meals daily will provide sufficient caloric intake without excess bulk. If not, the same quantity can be divided over five meals. No special food restrictions are indicated, except where specific idiosyncrasy exists. If further restrictions must be made, the addition of vitamin concentrates is advisable. One "fasting" day each week will often be found beneficial, and consists in limiting the food to water, broth, milk, and fruit juices. Although lack of bulk favors constipation, adequate adjustment seems to occur particularly with the aid of intestinal oxygenation.

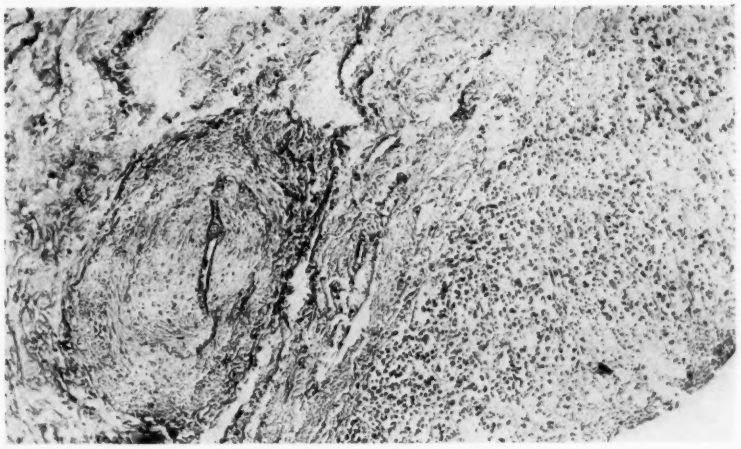

Fig. 4.—Extreme stenosis of lumen of a submucosal vessel. Note marked subintimal proliferation and several breaks in continuity of the elastica.

(c) *Intestinal aspiration.* By intermittent suction through the sigmoidoscope, distention can be relieved. It also appears to serve as a gentle internal massage to the bowel wall and tends to improve the tone of the musculature. This method is only employed at the beginning of therapy and is later completely replaced by intestinal oxygenation. It is essentially an office procedure carried out under direct vision. Relief is prompt and generally lasts for about twenty-four hours.

(d) *Intestinal oxygenation.* Intestinal oxygenation and the rationale of its employment have been described elsewhere.[16] Normally the large bowel is free or almost free of oxygen. It has been shown that following intestinal oxygenation, the gas is absorbed into and through the wall, increasing the oxygen tension within the blood plasma and the peritoneal cavity.[17] Conversely, the inhalation of oxygen increases the tension within the bowel lumen through the indirect hematogenous route. The bowel wall appears to serve as a semipermeable membrane, diffusion of the oxygen taking place either from the bowel lumen to the plasma or in the reverse direction. Man literally breathes through his intestine. The direct instillation of oxygen into the bowel lumen has been found most effective. It appears to improve the muscle tone and the elasticity of the wall. One of the most striking effects is the relief of constipation. Patients habituated to the use of enemas, who perhaps could

not move their bowels otherwise for three or four days, soon have daily formed bowel movements. The same effect is often noted in young patients. We have been able to demonstrate this effect in some patients suffering from constipation over a period of fifteen years without changing any other factor except the withdrawal of all cathartics and enemas. It is hardly conceivable that the presence of 200 c.c. of oxygen in a colon with a capacity of 2 to 3 liters has any direct mechanical effect by distention of the bowel wall, particularly since the gas is given very slowly. Another interesting observation in connection with the use of oxygen is the rapid disappearance of foul odor from the stool.

Fig. 5.—Apparatus for intestinal oxygenation, including small warming coil for immersion in basin of hot water or placing upon electric pad. A No. 16 soft rubber catheter is inserted into the rectum.

The oxygen dosage usually employed is approximately 200 c.c., one to three times daily. The apparatus pictured is set up in the bathroom, and the distal 3 or 4 inches of a No. 16 soft rubber catheter lubricated with K-Y jelly is inserted into the rectum. Administered at the rate of 90 bubbles per minute for ten minutes, it will deliver approximately 200 c.c. of oxygen at room temperature. Any similar device may be used. The gas is usually given with the patient in the upright position on the toilet seat, but in debilitated patients it may be administered in the recumbent posture. After proper instruction in the use of intestinal oxygenation, the patient is permitted to continue treatment at home. In some instances the dosage can eventually be cut down after one or two months or even discontinued except for periodic short intervals. During the ten years that intestinal oxygenation has been employed, no untoward result has been reported. In cases where the rectal route is not feasible, the oxygen may be administered by inhalation, though larger amounts are required to relieve intestinal atony.

In closing, it may be stated that no broad generalizations with regard to the therapeutic effects of oxygen are intended. Our experience has been sufficiently large, however, to warrant a rather conservative report so that others may be encouraged to judge of its usefulness.

SUMMARY

Intestinal vascular sclerosis is essentially a disease of old age. The chief symptoms are distention, constipation, indigestion, and abdominal cramps. Therapy consists of cardiovascular supportive measures, an easily assimilated diet, intestinal aspiration, and intestinal oxygenation.

I wish to acknowledge gratefully the assistance of Dr. I. Rothstein and Dr. W. H. Kastl in reviewing 1,000 necropsy protocols.

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MONOETHANOLAMINE (MONOLATE)

An Experiment on Its Antigenic Properties

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SINCE the beginning of treatment of varicose veins by injection, there has been a constant search for a more satisfactory sclerosing agent. At the present time sodium morrhuate is the most widely used solution. However, there are numerous reports of allergic reactions to sodium morrhuate, and several deaths, apparently due to allergic reactions, have been reported. These reactions could be expected because sodium morrhuate is made of the sodium salts of the fatty acids of cod-liver oil and is not protein free.

Recently, there has been marketed a solution of monoethanolamine oleate* which closely resembles sodium morrhuate in clinical use and results. Because this solution contains no protein, it was hoped that it would be free of the annoying and occasionally disastrous reactions of sodium morrhuate. A limited clinical trial has shown the material to be very satisfactory. During the period of clinical trial, a death was reported by Shelley,[1] which he ascribed to an allergic reaction to the injection of monolate. Recently, Golden and Heyerdale[2] have reported a case of sensitivity to both monolate and sodium morrhuate in the same individual. Although no other reports of allergic reactions or sensitivity to monolate have appeared in the literature, it was thought that a laboratory test of the antigenic properties of monolate would be desirable. The following experiments were then performed with a comparison of the antigenic action of 5 per cent sodium morrhuate and monolate.

REPORT OF EXPERIMENTS

Experiment 1. Each of three guinea pigs was injected intraperitoneally with 1 c.c. of 5 per cent sodium morrhuate. Three weeks later each was reinjected with 5 per cent sodium morrhuate. All showed a typical anaphylactic reaction. Two sneezed and became apprehensive; their hair raised, they had a slight convulsion, and recovered. The third had a similar reaction, with a more severe convulsion, and died.

Experiment 2. Each of 12 guinea pigs was injected intraperitoneally with 0.5 c.c. to 1.0 c.c. of monolate, depending on the size of the pig. Three weeks later each was given an intraperitoneal dose of 1.0 to 3.0 c.c. of monolate. There was no reaction in any guinea pig.

Experiment 3. Two cubic centimeters of monolate were injected into the ear vein of a rabbit. Three weeks later 2 c.c. of monolate again were injected intravenously. There was no reaction.

*Monolate.

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Experiment 4. Equal doses of 5 per cent sodium morrhuate and monolate were injected subcutaneously into a rabbit's ears. The size, intensity, and duration of the inflammatory reactions were practically identical.

Experiment 5. (a) Monolate gives a negative reaction to protein by the Greenberg method for total protein. (b) Monolate gives a positive biuret test. This is due to the monoethanolamine, and does not indicate the presence of protein. (c) Micro-Kjeldahl test is also positive. This is due to the presence of nitrogen in monoethanolamine, in the form of an amino group, and again does not indicate the presence of protein.

DISCUSSION

The results of this experiment were anticipated on the basis of the chemical compositions of the sodium morrhuate and the monolate. Sodium morrhuate is derived from cod-liver oil, and contains some protein, to which guinea pigs are readily sensitized and show a typical anaphylactic reaction. Monolate contains no protein and apparently will not sensitize guinea pigs. Although it is not possible on the basis of this small number of animals to make a final statement about the antigenic properties of monolate, it is encouraging to find that it is not a sensitizing antigen for any of this group of guinea pigs.

The similar inflammatory reaction produced on a rabbit's ear would reaffirm the clinical impression of a similarity of the sclerosing activity of the 5 per cent sodium morrhuate and monolate. Actually, in practice the monolate seems slightly more potent than sodium morrhuate, and to get a similar reaction, about two-thirds as much monolate as 5 per cent sodium morrhuate can be used.

Of course, no light has been thrown on the problem of drug sensitivity, and anyone who undertakes to give a large number of injections of any drug will sooner or later run across some person who is sensitive to that drug. At the present time no means of predicting such sensitivity has been determined. It has been my custom to give a very small injection of 0.2 c.c. to 0.3 c.c. at the time of the first injection, hoping to measure the patient's reactions to the injection and to the drug. This may be helpful.

CONCLUSIONS

No antigenic activity could be demonstrated experimentally in monolate. If it has any antigenic property, it is certainly much less antigenic than 5 per cent sodium morrhuate, which it closely resembles in application and results. Monolate is, therefore, a more satisfactory agent for use in the injection treatment of varicose veins.

I wish to express my thanks to Dr. Paul Micheal for performing the laboratory test and for the use of his laboratory facilities for the animal experimentation.

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MULTIPLE PRIMARY MALIGNANT LESIONS*

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MANY reports of multiple primary malignant lesions in the same persons have appeared in the literature since Billroth published the first case in 1869. The first American author to become interested in the problem was Major who, in 1918, reviewed the literature, which was chiefly contributed by German authors. Subsequently Owen (1921), Ophüls (1926), Hanlon (1931), Warren and Gates (1932), Hurt and Broders (1933), Schreiner and Wehr (1934), Bugher (1934), Burke (1936), Austin (1938), Kirshbaum and Shively (1938), and Stalker and others (1939) have contributed materially to the American literature by publishing large series of cases. In addition, many authors have contributed isolated cases or small series of cases. The summaries of the larger series of cases may be found in Tables I, II, and III where the several series are broken down into small groups because of variations in methods of diagnosis and, particularly in the autopsied and surgical cases, because of the variation in sex incidence and the types of lesions encountered. The Bellevue cases conform in general with those of the quoted autopsy cases.

The Bellevue Hospital material was selected from 6,836 consecutive autopsies performed in the four divisions of the hospital during the past eight years. In 1,044, or 15.2 per cent, of these autopsies malignant lesions were diagnosed, and in 21 of the 1,044 autopsies, or 2.0 per cent, more than one independent malignant lesion was diagnosed. The 21 cases here presented were selected after a review of the autopsy protocols, the microscopic sections, and the clinical histories. The cases are not limited to carcinomas, but include all types of malignant lesions.

The microscopic sections meet the following criteria:

a. Each lesion possesses generally recognized malignant characteristics.

b. Each lesion has the histologic pattern commonly associated with malignant lesions arising from the tissue or organ in question.

c. Each lesion is a distinct entity, metastases having been considered and ruled out in so far as possible in each instance. These criteria closely follow those established by Warren and Gates, and, like theirs, exclude an additional criterion, namely, that each tumor must produce its own metastases, which was advocated by Billroth but which has since been generally discarded.

The clinical histories were studied with a view to establishing the relationship of the duration of the individual lesions and collecting data relative to the familial tendency to malignant diseases. Investigation along both these

*From the Department of Pathology, Bellevue Hospital, New York.
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TABLE I

AUTOPSY SERIES

(All Diagnoses Confirmed by Histologic Study)

AUTHOR	MALIGNANT CASES	MULTIPLE MALIGNANT CASES	PER CENT	AVERAGE AGE	RATIO M:F
Ophüls	512*	15	2.9	--	--
Hanlon	950	18	1.9	62.6	13:5
Warren and Gates	1,078	40	3.7	61.8	15:25
Bugher	983	30	3.1	61.0	24:6
Burke	583	46	7.8	--	35:11
Austin	887	24	2.7	--	--
Kirshbaum and Shively	1,411	25	1.77	63.3	17:8
Tullis	1,044	21	2.0	58.4	17:4

*This total includes 11 patients with Hodgkin's disease and 23 patients with acute and chronic leucemia. Seven patients with glioma listed elsewhere are not included in this total.

TABLE II

SURGICAL SERIES

(All Diagnoses Confirmed by Histologic Study)

AUTHOR	MALIGNANT CASES	MULTIPLE MALIGNANT CASES	PER CENT	AVERAGE AGE	RATIO M:F
Hurt and Broders	2,124	71	3.3	50.4	32:39
Stalker and co-workers	2,500 (approx.)	113	4.52	59.7	50:63

TABLE III

CLINICAL SERIES
(CLINICAL CASES "OBSERVED")

(All Diagnoses Not Confirmed by Histologic Study)

AUTHOR	MALIGNANT CASES	MULTIPLE MALIGNANT CASES	PER CENT	AVERAGE AGE	RATIO M:F
Owen	3,000	143	4.7	–	–
Ward	1,773	94	5.3	–	55:39
Schreiner and Wehr	11,212	307	2.7	–	–

lines was disappointing. Frequently, unless one of the lesions was on the body surface, ante-mortem diagnosis of the second lesion was not made. As for the familial tendency, in only 3 cases (Nos. 1, 17, 19), or 14 per cent, was there a positive family history of cancer. This is slightly below the figures of 15 to 30 per cent usually reported. However, in one case the causes of death of the patient's family were unknown to him, and in 2 others this information was not obtained before the patients' death. If these 3 cases are subtracted from the total, the percentage of positives becomes 16.6. Even this is believed to be an understatement, since the typical Bellevue patient is drawn from a large city with a large immigrant population. Often he has an imperfect knowledge of English; more often he has lost contact with his family in the fatherland.

Realizing that errors of omission are as unfortunate as errors of commission, a conscious effort to avoid both was made. It was felt that lesions with a known tendency to multicentric origin, such as multiple parenchymal cell carcinomas

of the liver, and lesions of possible malignant origin, which are not yet universally accepted as such, including the leucemias and Hodgkin's disease, should be excluded from this series. Also two cases of bilateral hypernephromas of identical histologic pattern, where it was impossible to rule out the possibility of one being a metastasis from the other, and one case of bilateral carcinomas of the ovaries, where similar conditions prevailed, were not included. However, one case of bilateral carcinomas of the female breasts was included because the one had been surgically removed four years before the appearance of the other, and the two were of different histologic pattern.

TABLE IV

NO.	SEX	AGE	TYPE LESION	ORGAN	METASTASIS	TYPE LESION	ORGAN	METASTASIS
1	F	58	Perithelial sarcoma	Liver	0	Adenocarcinoma	Uterus	0
2	M	44	Bronchiogenic carcinoma	Lung	x	Squamous-cell carcinoma	Scrotum	0
3	F	73	Parenchymal-cell carcinoma	Liver	0	Fibrosarcoma	Pancreas	x
4	M	68	Squamous-cell epithelioma	Esophagus	0	Adenocarcinoma	Prostate	0
						Spindle-cell sarcoma	Stomach	x
5	M	34	Squamous-cell carcinoma	Bile ducts	0	Adenocarcinoma	Stomach	x
6	M	56	Adenocarcinoma	Colon	x	Adenocarcinoma	Prostate	0
7	F	33	Duct-cell carcinoma	Right breast	0	Colloid carcinoma	Left breast	x
8	F	60	Adenocarcinoma	Kidney	x	Basal-cell epithelioma	Cervix	0
9	M	40	Colloid adenocarcinoma	Rectum	x	Papillary adenocarcinoma	Transverse colon	x
10	M	66	Bronchiogenic carcinoma	Lung	x	Adenocarcinoma	Prostate	0
11	F	58	Adenocarcinoma	Rectum	x	Papillary adenocarcinoma	Rectum	x
12	M	76	Adenocarcinoma	Prostate	0	Adenocarcinoma	Pancreas	0
13	M	51	Adenocarcinoma	Stomach	x	Parenchymal-cell carcinoma	Liver	x
14	M	54	Bronchiogenic carcinoma	Lung	x	Squamous-cell epithelioma	Tongue	0
15	M	63	Adenocarcinoma	Rectum	0	Basal-cell epithelioma	Temple	0
16	M	50	Parenchymal-cell carcinoma	Liver	0	Adenocarcinoma	Stomach	x
17	M	58	Hypernephroma	Kidney	x	Adenocarcinoma	Pancreas	0
18	M	65	Adenocarcinoma	Stomach	0	Adenocarcinoma	Stomach	0
19	M	75	Adenocarcinoma	Stomach	0	Squamous-cell epithelioma	Esophagus	0
20	M	70	Adenocarcinoma	Stomach	0	Adenocarcinoma	Ascending colon	0
21	M	74	Adenocarcinoma	Stomach	0	Adenocarcinoma of polyp	Stomach	0
						Adenocarcinoma of polyp	Stomach	0

The cases are listed as a group in Table IV. There are 21 cases, representing 44 lesions, of which 3 are sarcomas and 41 carcinomas. Tables V, VI, VII, and VIII show the organs or organ systems most frequently involved.

The gastrointestinal system is most frequently involved. Twelve cases (57 per cent), representing 20 lesions, are listed under this category. Eight of these 12 cases show 11 lesions in the stomach. Only 2 lesions involve the skin pri-

marily, although 6 lesions arise from stratified squamous epithelium. This distribution is in accord with that in other autopsy series, but is in complete disagreement with the distribution in the surgical and clinically observed cases. In the surgical and clinical series stress is laid on the predominance of skin neoplasms among multiple malignant lesions. Fifty-eight of the 152 lesions among 71 surgical cases reported by Hurt and Broders originated in the skin. Thirty-five of the 113 cases reported by Stalker and associates involved only the skin. Ward, working in England, who reported only rodent ulcers, states that 5.3 per cent are multiple. Since these lesions are by virtue of their location discovered readily and early, they are frequently successfully treated before the patients come to autopsy. If there is a change of hospitals in the interim, the foreign clinical records may be lost to the pathologist.

TABLE V

CASES IN WHICH AT LEAST ONE OF THE LESIONS IS PRIMARY IN THE GASTROINTESTINAL TRACT

CASE NO.	SEX	AGE	LESION	ORGAN	LESION	ORGAN
4	M	68	Spindle-cell sarcoma	Stomach	Squamous-cell epithelioma	Esophagus
					Adenocarcinoma	Prostate
5	M	34	Adenocarcinoma	Stomach	Squamous-cell carcinoma	Bile ducts
13	M	51	Adenocarcinoma	Stomach	Parenchymal-cell carcinoma	Liver
16	M	50	Adenocarcinoma	Stomach	Parenchymal-cell carcinoma	Liver
18	M	65	Adenocarcinoma	Stomach	Adenocarcinoma	Stomach
19	M	72	Adenocarcinoma	Stomach	Squamous-cell epithelioma	Esophagus
20	M	70	Adenocarcinoma	Stomach	Adenocarcinoma	Ascending colon
21	M	74	Adenocarcinoma	Stomach	Adenocarcinoma of polyp	Stomach
					Adenocarcinoma of polyp	Stomach
6	M	56	Adenocarcinoma	Colon	Adenocarcinoma	Prostate
9	M	40	Colloid adenocarcinoma	Rectum	Papillary adenocarcinoma	Transverse colon
11	M	58	Adenocarcinoma	Rectum	Papillary adenocarcinoma	Rectum
15	M	63	Adenocarcinoma	Rectum	Basal-cell epithelioma	Temple

The liver is involved in 5 cases, the prostate in 4, the pancreas in 3, the lung in 3, the uterus in 2, and the kidney in 2.

Two cases have 3 primary malignant lesions. Case 4 shows involvement of the prostate (adenocarcinoma), the middle third of the esophagus (squamous-cell epithelioma), and the stomach (spindle-cell sarcoma). Case 21 shows involvement of two distinct polypi in the fundus of the stomach (adenocarcinomas) and an infiltrating lesion in the pylorus (adenocarcinoma).

In 14 cases there are metastases from at least one of the lesions, and in 3 cases from both of the lesions. In 7 cases no metastases could be found.

Seventeen patients were males and 4 were females, a ratio of 4.2:1. Except in one instance (Warren and Gates), the published autopsy series show a comparable ratio of males to females. In the surgical series, however, the ratio

is slightly in favor of the females. The variation may be explained in part by the fact that while women are willing to submit to operation, it is more difficult to obtain permission for post-mortem examination of the female body.

TABLE VI

CASES IN WHICH ONE OF THE LESIONS ARISES FROM STRATIFIED SQUAMOUS EPITHELIUM

CASE NO.	SEX	AGE	LESION	ORGAN	LESION	ORGAN
2	M	44	Squamous-cell epithelioma	Scrotum	Bronchiogenic carcinoma	Lung
4	M	68	Squamous-cell epithelioma	Esophagus	Spindle-cell sarcoma	Stomach
					Adenocarcinoma	Prostate
8	F	60	Basal-cell epithelioma	Cervix	Adenocarcinoma	Kidney
14	M	54	Squamous-cell epithelioma	Tongue	Bronchiogenic carcinoma	Lung
15	M	63	Basal-cell epithelioma	Temple	Adenocarcinoma	Rectum
19	M	72	Squamous-cell epithelioma	Esophagus	Adenocarcinoma	Stomach

TABLE VII

CASES IN WHICH ONE OF THE LESIONS IS PRIMARY IN THE LIVER OR BILE DUCTS

CASE NO.	SEX	AGE	LESION	ORGAN	LESION	ORGAN
1	F	58	Perithelial sarcoma	Liver	Adenocarcinoma	Uterus
3	F	73	Parenchymal-cell carcinoma	Liver	Fibrosarcoma	Pancreas
5	M	34	Squamous-cell carcinoma	Bile ducts	Adenocarcinoma	Stomach
13	M	51	Parenchymal-cell carcinoma	Liver	Adenocarcinoma	Stomach
16	M	50	Parenchymal-cell carcinoma	Liver	Adenocarcinoma	Stomach

TABLE VIII

CASES IN WHICH ONE OF THE LESIONS IS PRIMARY IN THE PROSTATE

CASE NO.	SEX	AGE	LESION	ORGAN	LESION	ORGAN
4	M	68	Adenocarcinoma	Prostate	Squamous-cell epithelioma	Esophagus
					Spindle-cell sarcoma	Stomach
6	M	56	Adenocarcinoma	Prostate	Adenocarcinoma	Transverse colon
10	M	66	Adenocarcinoma	Prostate	Bronchiogenic carcinoma	Lung
12	M	76	Adenocarcinoma	Prostate	Adenocarcinoma	Pancreas

The average age in this series is 58.4 years. The ages vary from 33 to 75 years, the ages being those at time of death. This average conforms with other published reports. It is slightly above the generally accepted age incidence for single malignant lesions, thereby lending credence to the clinical rule that those tumors which appear late in life are likely to be less rapidly lethal than those which appear early. Being less rapidly lethal, they do not cause the death of the host before a second lesion has time to develop.

COMMENT

The amassed data from several large series of autopsies from American institutions reveal that in 7,448 cases with malignant lesions there are 219 cases (2.8 per cent) with multiple malignant lesions. Kirshbaum and Shively, on the basis of their individual data, conclude "individuals may be endowed with a congenital, or acquired, predisposition toward tumor formation." Warren and Gates, and Austin, conclude that the incidence of multiple malignant lesions is

higher than can be explained on the basis of chance alone. A mathematical formula for computing the expected incidence of multiple malignant tumors when compared with the actual facts leads Bugher to conclude that the incidence is "equal to or greater than may be expected from chance alone." Although the percentage of multiples in the Bellevue series is somewhat below that of the total published series combined, the facts seem to warrant concurrence with those whose opinion it is that multiple malignant lesions occur more frequently than can be explained by chance alone. It seems fair to predict that as the life expectancy of patients with cancer is increased due to refinements in diagnosis and treatment, the incidence of multiple malignant tumors will increase and lend more convincing evidence in favor of the foregoing conclusion.

In reviewing the large number of autopsies necessary for the preparation of a paper of this nature, the frequency with which one encounters benign tumors in association with malignant tumors is striking. Studied in connection with family history, the problem is intriguing. It brings up the question of the interrelationship of hereditary predisposition for tumor formation and reaction to environment, and how that interrelationship may affect the health and happiness of the individual. The answer to the riddle is not yet apparent, but the possibility of theory becoming fact should interest the clinician now. With this information in mind he may help his patient and himself in two ways: first, in realizing that his diagnosis is not complete until he has ruled out the possibility of a second tumor once one is found; and, second, in realizing, as Stalker and co-workers have emphasized, the importance of free discussion and investigation of the other members of the family of a cancer patient for the possible presence or development of malignant disease.

SUMMARY

1. Twenty-one autopsies with multiple malignant lesions occurring in 1,044 autopsies with malignant lesions are reported. This is an incidence of 2.0 per cent.

2. There were 17 male patients and 4 females, with a combined average age incidence of 58.4 years.

3. Multiple malignant lesions are believed to occur more frequently than can be explained on a basis of chance alone.

4. The clinician is urged to rule out the possibility of a second tumor once one has been diagnosed.

5. The clinician is urged to discuss freely with the family of his patients with cancer the possible familial tendency of the disease.

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CONGENITAL ABSENCE OF THE GALL BLADDER AND CYSTIC DUCT*

Report of a Case

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CONGENITAL anomalies of the gall bladder and the biliary ducts have been reported with increasing frequency in the past two decades, but they still remain relatively rare conditions. The importance of these structures in medicine and surgery warrants the description of these anomalies, which may at times assume major significance in diagnosis and treatment.

REPORT OF A CASE

S. L. (10922-1939), a 76-year-old white male, was admitted to the Metropolitan Hospital on Oct. 17, 1939, with complaints referable to the urinary system. He had received treatment for a mild diabetic condition, but had otherwise been in good health up to the present illness. The family history was irrelevant. There were no gastrointestinal symptoms, except for anorexia and constipation which had developed concomitantly with his urinary complaints.

After physical examination and laboratory work-up, the diagnosis was made of: (1) Fibrosis of prostate with contraction of the bladder neck. (2) Diabetes mellitus. (3) Arteriosclerotic heart disease—compensated. First-stage prostatectomy was performed on Nov. 8, 1939. The patient never rallied following the operation, but went steadily downhill despite all therapeutic measures. He developed an ascending urinary infection, and died Jan. 12, 1940.

During the course of the autopsy it was noted that the gall bladder and cystic duct could not be located. Acquainted with the rarity of this anomaly, we centered our attention on this focus. Careful examination of the skin of the anterior abdominal wall and of the underlying peritoneum did not reveal any scars. The site usually occupied by the gall bladder was free of adhesions or fibrous tissue, which may sometimes conceal an atrophic or shrunken gall

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bladder. The extrahepatic biliary ducts were carefully dissected and their continuity was traced into the duodenum. There were neither signs nor vestige of a cystic duct.

The liver weighed 1,430 Gm. and appeared normal as to shape and relations to the adjacent viscera. The normal markings of the gall bladder fossa were effaced. Glisson's capsule was thickened, and the surface of the liver was finely granular. The organ was firm and cut with resistance. The cut edges were sharp and distinct. The intrahepatic biliary ducts were moderately dilated and filled with viscid green bile mixed with granular debris. The right and left hepatic ducts issued from the transverse fissure, and immediately united to form a dilated common hepatic duct, which was 8 cm. long and had an average diameter of 1 cm. This duct was filled with viscid green bile and showed a small bulbous dilatation 0.5 cm. proximal to its entrance into the duodenum. The duodenal papilla appeared normal. Multiple sections of the liver failed to reveal any trace of an intrahepatic gall bladder. Careful examination of the common hepatic duct did not show any evidence of a cystic duct. The cystic artery was absent.

Microscopically, the capsule was irregularly thickened, and there was extensive subcapsular lymphocytic infiltration. The lobules were irregular in shape, but the relation of the central vein to the lobule was preserved. The perilobular connective tissue was increased and was infiltrated with lymphocytes, plasma cells, and occasional polymorphonuclear leucocytes. There was a proliferation of biliary canaliculi. The larger ducts were dilated and filled with plugs of bile. The hepatic parenchyma, for the most part, appeared normal. However, some cells, especially those at the periphery of the lobules, showed degenerative phenomena and some contained bile pigment. The blood vessels showed thickened walls and were engorged with blood. Sections of the capsule at the site usually occupied by the gall bladder failed to show any evidence of that structure.

The pancreas weighed 78 Gm. and was somewhat indurated. Microscopically, there was a slight increase in the interstitial connective tissue. The islets were abundant and appeared well preserved.

DISCUSSION

The significance of anatomic abnormalities of the gall bladder and biliary passages becomes increasingly important as our knowledge of this system progresses. It has been estimated that in at least 10 per cent of all autopsies, some anomaly of the gall bladder, bile ducts, or adjacent blood vessels can be demonstrated.

Agenesis of the gall bladder and cystic duct has been described as an independent finding in about 40 cases. More commonly, this anomaly is found associated with other abnormalities of the hepatobiliary system. A total of about 60 cases have been described where absence of the gall bladder and cystic duct are accompanied by other structural defects of this system.

In 1928 Bower,[1] in an extensive review of the anomalies of this system, listed absence of the gall bladder and cystic duct as the fourth most rare anomaly of the hepatobiliary system. He cited 31 case reports of congenital absence of the gall bladder and cystic duct occurring independent of other

anomalies. In that same year, Alglave[2] added another case to this series. Since then cases have been reported, among others, by Danzis,[5] Tallmadge,[6] and Robertson and associates.[7] Knox[8] has reported two cases in a series of 2,000 autopsies. Mentzer[9] and Nagel have seen the condition once each in a series of 1,600 autopsies. Emmert[4] in 1931 reported the first case he had seen in twenty years of experience. Mackmull[3] in 1930 reported the first case encountered by Schaeffer in 3,000 dissections. This case represents the first instance encountered at the Metropolitan Hospital in a series of 3,660 consecutive autopsies.

If the gall bladder is not found at autopsy or at operation, four possibilities must be borne in mind: (1) The gall bladder has been previously removed. (2) The gall bladder, due to repeated attacks of cholecystitis, has become atrophic or is buried in adhesions. (3) The gall bladder is intrahepatic or is otherwise abnormally located. (4) The gall bladder is congenitally absent.

In the case under discussion there was no history of any attacks of cholecystitis, nor of a cholecystectomy. There were no scars on the anterior abdominal wall nor on the peritoneal surface of the abdominal wall. There were no adhesions nor scar tissue at the gall bladder area. Multiple sections of the liver revealed no evidence of an intrahepatic gall bladder, nor could it be found in any other site. The only possibility that remained was that the gall bladder was congenitally absent.

The gall bladder and cystic duct are normally absent in certain species of animals (horse, mule, elephant). Some species may or may not have it (giraffe). In certain animals (pigeon) a gall bladder is present in embryonic life but disappears in later development.[9] Its absence in man has been regarded by some writers as atavistic.

Congenital anomalies of the hepatobiliary system are of importance to the surgeon and clinician as well as to the pathologist and anatomist. These anomalies may lead to erroneous diagnoses, as when the gall bladder fails to visualize on cholecystography. They may also give rise to technical difficulties in surgical procedures. We recently had the opportunity to see a case where a cholecystogastrostomy was performed for common duct obstruction. The operation was technically successful, but at autopsy we found that the cystic duct ran an independent course up to immediately before the ampulla of Vater. There was not sufficient intraductile pressure to force the accumulated bile from the common duct up the long cystic duct into the gall bladder, and through the stoma between the gall bladder and the stomach. Had this anomaly been recognized at operation, an attempt might have been made to anastomose the widely dilated common hepatic duct to the stomach or duodenum, thereby prolonging the life of the patient.

Dilated bile ducts often, but not constantly, are found associated with absence of the gall bladder. Waring[10] was the first to make the observation that "when the gall bladder and cystic duct are absent, the common duct is usually found to be enlarged a short distance above the entrance into the duodenum, so as to form a fusiform sac, which may serve to a certain extent, as a substitute for the gall bladder in the storage of bile which is secreted between the periods of digestion." Such a localized dilatation was found in this case in an already dilated common hepatic duct. It is interesting to note

that in agreement with Bower's statistics, the deceased showed dilated ducts and had lived to 76 years of age. Bower[1] states the average age of those who died with normal ducts was only 36, as compared with 52 years for those whose hepatic ducts were dilated. This is in accord with the fact that in some species of animals that are not provided with a gall bladder, the common duct dilates to compensate.

CONCLUSION

1. Congenital absence of the gall bladder and cystic duct is a relatively rare entity.

2. A case is reported where this condition was found at autopsy, but there were no symptoms clinically to indicate the presence of this anomaly.

3. Anomalies of the hepatobiliary system are of prime importance in the medical sciences.

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OBSERVATIONS ON CAPILLARY PERMEABILITY AND INFLAMMATION IN THE SKIN OF SENSITIZED RABBITS*

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TRYPAN blue when given intravenously to normal rabbits localizes and concentrates in areas of inflammation only during a specific interval. This interval is not determined by the presence of edema, hyperemia, or polymorphonuclear leucocytes.[1-3] It has been suggested that this colloidal dye localizes and concentrates in areas of inflammation as a "result of a temporary change which occurs in the epithelial cells, the connective tissue cells, and the endothelial cells of the capillaries. This change in the permeability of these cells results from the effect of xylol on the epithelial cells. The normal metabolism of the epithelial cells is disturbed, as shown by the presence of pyknosis, karyorrhexis, and necrosis. The results of the effects of this abnormal intercellular fluid on the capillary endothelium is, no doubt, a compensatory mechanism, permitting the passage of water and crystalloids at a more rapid rate than normal and also readily allowing the passage through the vessel wall of the cells, colloids, and plasma proteins."[4]

Ramsdell[5] injected trypan blue intravenously to demonstrate the immediate skin reaction in rabbits and guinea pigs made hypersensitive to horse serum. The dye was injected intravenously immediately before 0.01 c.c. of horse serum was given intradermally. The infiltration of dye into these reactive sites, according to Ramsdell, "is at present to be looked upon as no more than a sort of indicator of the presence of an edematous change which is an expression of a toxic action upon the capillary endothelium." In discussing the observations of Ramsdell, Burrows[6] states that "it would be of interest to know the results if these experiments were repeated with controls."

The present paper is first a report of our observations on the localization of trypan blue in the skin of normal and sensitized rabbits following the intradermal injection of horse serum, and secondly, our observations on the effect of trypan blue on the development of the local reaction following an intradermal injection of horse serum in the skin of sensitized rabbits.

METHODS AND MATERIALS

The skin of the rabbits is carefully shaven twenty-four hours or longer before the horse serum† is injected intradermally. The serum is injected at

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varying intervals before 10 c.c. of a 0.2 per cent solution of trypan blue is given intravenously. The skin is observed for an hour for the appearance of a blue color.

A series of intravenous and intraperitoneal injections of horse serum is given for the production of sensitivity. The serum, obtained by bleeding from the heart, is titrated for precipitins in those rabbits used to study the effect of trypan blue on the skin reactions. The horse serum for intradermal injection is diluted with saline.

EXPERIMENTAL

Localization of Trypan Blue in Areas of Skin Injected With Horse Serum. Normal and Sensitized Rabbits.—Eight normal rabbits are injected intradermally with 0.2 c.c. of horse serum at the following intervals before 10 c.c. of a 0.2 per cent solution of trypan blue is given intravenously; twenty-four hours, five hours, three hours, one hour, thirty minutes, and immediately. In the normal rabbits only the last two areas, where the serum is injected immediately before the dye is given, become blue within a period of thirty minutes. In a group of six sensitized rabbits the dye localizes and concentrates in the areas of skin injected with the horse serum as long as three hours before the dye is given.

The greatest amount of dye concentrates in the areas injected with the serum the shortest time before the dye is given. A larger area of blue occurs at the site of the intradermal injection in the sensitized rabbits than in the normal rabbits.

There is only a small amount of edema in the skin at the site of the intradermal injections of the serum in the normal rabbits. In contrast to this, there is extensive edema and hyperemia in the skin where the horse serum is injected in the sensitized animals.

These observations show that a similar change occurs in the skin of both the normal and the sensitized rabbit following the intradermal injection of horse serum which permits the localization and concentration of trypan blue. This change persists longer, however, in the sensitized animals than in the normals. The localization and the concentration of this dye are not determined by either the presence or the absence of edema and hyperemia. There are considerable edema and hyperemia in the area where horse serum is injected twenty-four hours previous to the injection of the dye in the sensitized rabbit. The dye fails, however, to concentrate in the area. These observations seem to support the idea previously expressed that trypan blue localizes and concentrates in areas as a result of a change in the tissue cells. This change is a temporary one. The capillary endothelial cells, the tissue cells, and the epithelial cells are all apparently affected.

Effect of Trypan Blue on the Development of the Local Reaction in the Skin of Sensitized Rabbits.—It seems of interest in this experiment to study the effect of trypan blue on the development of the local reaction in sensitized animals. The results of the preceding experiments support the opinion previously expressed that trypan blue localizes and concentrates in areas of inflammation as a result of a temporary change in the endothelial cells, the con-

nective tissue cells, and the epithelial cells.[4] Moon[7] thinks that the above cells become sensitized and are injured directly by contact with the specific antigen. The presence of a colloid in these cells might influence their response to a specific antigen.

Five rabbits sensitized to horse serum are used in this experiment. Five days before the intradermal injection of the horse serum, the precipitin titer of the serum from these rabbits varied from 1:320 to 1:5,120. Ten cubic centimeters of a 0.2 per cent solution of trypan blue are injected intravenously into each of three of these rabbits, and into one control thirty hours and again six hours before the intradermal injection of 0.1 c.c. of the following dilutions of horse serum: undiluted, 1:10, 1:20, 1:40, 1:80, and 1:100. The skin is dark blue when the horse serum is injected. There are edema and hyperemia after twenty-four hours in each area of skin injected with the serum diluted as much as $\frac{1}{40}$ in each of the sensitized rabbits, while only a small reaction is present in the normal rabbits where undiluted horse serum is injected. The skin in the two sensitized rabbits without dye corresponds exactly with the reaction in the three rabbits given the trypan blue.

It may be concluded from this experiment that trypan blue does not affect the reaction of sensitized cells to a specific antigen.

DISCUSSION

The observations made in this experiment that trypan blue following an intravenous injection localizes and concentrates only during a specific interval following the intradermal injection of horse serum corresponds to other studies where this dye was found to localize and to concentrate in areas of inflammation produced by the local application of xylol only during a specific interval.[1, 2] The present experiment also shows that the time in which trypan blue localizes and concentrates in an area is not determined by the presence of edema and hyperemia. The localization and the concentration of this colloidal dye in specific areas of skin supports the opinion that "the connective tissue and the vascular system differs as far as their functional state is concerned in sensitized and non-sensitized animals."[8]

There are many technical difficulties that one encounters in an attempt either to saturate or to block and then to study the reaction of specific cells. Cannon and his associates,[9] and Jaffe[10] have discussed these in considering the effect of blocking the reticulo-endothelial system on the formation of antibodies. The occurrence of edema and hyperemia at the site of the injection of the antigen in the sensitized rabbits given trypan blue similar to that occurring in rabbits without this dye certainly supports the opinion that "the storage of the dye does not affect the vital parts of the cells and in the dye-filled cells sufficient cytoplasm is left to carry on its metabolic processes. The cell also is able to take in more foreign material of the same or of another kind."[10]

These observations show that trypan blue localizes and concentrates in areas of skin injected with horse serum in sensitized rabbits during a specific interval. Furthermore, the greatest quantity of dye localizes and concentrates

in the area of skin injected for the shortest interval before the dye is given. These results suggest that immediately following the contact of the antigen with the sensitized cells a change occurs in the cells which permits the entrance of the colloidal dye. This change in the cell is transient. If the cell is completely destroyed, the coagulated cytoplasm may become blue by a diffusion of the colloidal dye from the intercellular fluid. In the rabbits where extensive injury occurs following the intradermal injection of horse serum, trypan blue may be observed to localize at the periphery of the lesion for as long as twenty-four hours. The localization of dye at the periphery of such a lesion must be differentiated from the localization of the dye which occurs throughout an area infiltrated by the horse serum. In the former, the cells are affected by being proximal to severely injured tissue, while in the latter the localization and the concentration of dye apparently results from the effects produced by the contact of the antigen with sensitized cells. It must be remembered that the metabolism of a cell may be disturbed by many different processes.

The Arthus' phenomenon is not influenced by the intravenous injection of ink given before and after sensitization.[10] Although the reaction produced by the intradermal injection of horse serum into a sensitized rabbit may be different from the Arthus' phenomenon, it is of interest to know that trypan blue and ink do not affect the development of either the hypersensitive reaction or the Arthus' phenomenon.

SUMMARY

Trypan blue following an intravenous injection localizes and concentrates in areas of the rabbit's skin injected with horse serum if the serum is injected a short time before the dye is given. The time during which this dye localizes and concentrates in the skin is much longer in sensitized rabbits than it is in the skin of normal rabbits.

The localization and the concentration of trypan blue in areas of inflammation produced by horse serum are not determined by the presence of edema and hyperemia. This dye may not concentrate in all areas of edema and hyperemia, as shown by the failure of the dye to concentrate in areas of skin injected with horse serum twenty-four hours previously to the injection of the dye.

The presence of trypan blue in the tissues of a sensitized rabbit apparently does not affect the subsequent reaction that occurs when the antigen is injected intradermally.

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PERCUSSION AND AUSCULTATORY PERCUSSION WITH A NEW INSTRUMENT*

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IN ASCERTAINING the condition of the lungs, the size of the heart, great vessels, and viscera, no clinical method will ever have the finality of the roentgen ray. However, clinical means of evaluation of these facts, such as percussion and auscultation, are very important in the everyday practice of medicine, and any instrument that might improve our ability to recognize variations from normal justifies its existence. Moreover, competent evidence exists that percussion and its modifications can be fairly exact if done carefully.[1]

Ever since Auenbrugger first published his results obtained from percussion in 1761, there have been attempts at variation of this clinical method.[2] The plexímeter was first invented by Piorry of Paris in 1828, and has been constantly improved since then. During the past three years, after working with several hollow plexímeters in order to magnify the sound conduction, I selected one which gave very satisfactory results. It consists of an instrument called the Fox localizing attachment, whose original purpose was for localizing cardiac murmurs in auscultation of the heart. For its new use it has been renamed the resonant plexímeter. The instrument consists of a localizing rod attached to a resonating chamber or sounding box, and is used in percussion instead of the plexímeter finger. The use of the resonant plexímeter in percussion of the chest, the heart borders and great vessels, gave a more discernible change between resonance, flatness, and dullness, than was possible without this aid.

While experimenting with this instrument, it was attempted to lead off the sound obtained from percussion directly to the ears of the examiner; in other words, to employ auscultatory percussion. It was found at first that the sound reaching the examiner's ears was too loud to be analyzed accurately. A plug of cotton inserted into the instrument where it is attached to the stethoscope tubing, sufficiently muffled the sound so that it can be properly evaluated. Loudness is not a criterion in its use. In the determination of the size of heart, it is preferable to start in the center of the anterior chest wall over the sternum and lightly percuss the plexímeter as it is slowly moved in each direction, until a change in quality of the sound is noted. Care must be taken to

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tap lightly with the ball of the finger, and to keep the strokes of the finger of approximately the same intensity. Care must also be taken that the localizing rod is between the ribs and not on the ribs, because the ribs conduct and emit sound differently than do the soft tissues. In our opinion other organs, such as the liver, spleen, consolidated areas in the chest and pleural effusions can also be percussed with fair accuracy. In the case of the liver, the instrument is placed over the apparent center of the liver anteriorly in the midclavicular line, and percussion is employed in each direction until a definite change in the quality of sound is elicited.

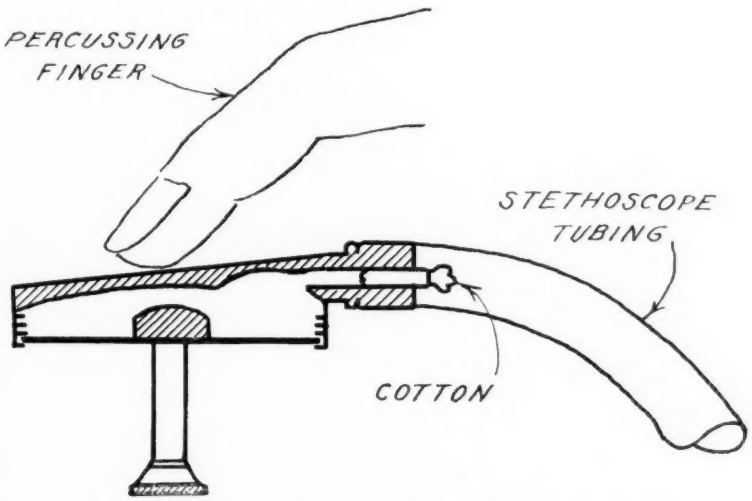

Fig. 1.—The resonant plexímeter for auscultatory percussion.

Certain difficulties inherent in the entire study of percussion must be considered at this point, so that any method may be properly evaluated.[3] It must be understood that the word resonance can mean all things to all men. It cannot be accurately described. Each individual has his own standard for comparison. What is important is that the interpretation based on this standard should be the same in each case. To achieve this, nothing will take the place of practice. The percussion sound is produced by successive layers of the chest; a light stroke will bring into vibration the superficial layers and organs, and a heavy stroke the deeper layers. The plexímeter finger or instrument must be placed fairly snugly against the chest wall so as to damp the vibrations of the chest wall and bring out the vibrations of the tissue that we intend to percuss. Each percussion note consists of a predominant or fundamental tone and overtones. To evaluate the percussion note properly, we have to disregard the overtones. The percussion note also depends on such seemingly insignificant aspects, such as the pedestal on which the subjects rest, e.g., whether the subject stands on a stone floor or rests in bed. In the former case, there is a diminution of overtones; in the latter, an accentuation. It would, therefore, be better to have the subject standing or sitting on a hard object, rather than lying on a soft bed. This is a very important factor in reducing the number of overtones. The fundamental or predominant tone has several character-

istics, such as pitch and resonance. The pitch is determined by the rate of vibration of the organs percussed. If the rate of vibration is high, the pitch is high. The resonance of a body depends on its ability to keep vibrating when struck. If the body stops vibrating after it is struck, it is nonresonant. The pitch and resonance both depend on the tension, thickness, extent, and constitution of the matter of the body that is vibrating. The tension and thickness for example might be different in the same pathologic conditions in different individuals; thus a pneumothorax note may be of high or moderate pitch, depending on the tension in the pleural cavity.[3] It can thus be seen that no instrument can possibly take the place of careful analysis of the percussion note.

HISTORICAL

The historical development of auscultatory percussion is interesting, because it shows how widely different the opinions of reliable observers can be as to its validity.[4] The instrument here suggested, we believe, circumvents the difficulties experienced with the older instruments, because it utilizes a direct method of percussion, and an evaluation of this percussed area by auscultation. The following historical sketch will show the difficulties inherent in this system of clinical diagnosis, and will show how some of these have been surmounted.

Auscultatory percussion was first suggested by Laënnec in his *Traite de l'auscultation mediate*,[5] but he did not use it to determine the size of the heart. Cammann and Clark[6] were the first actually to attempt to determine the size of the heart. One end of a rod was placed against the chest wall of the subject and the other in the ear of the examiner. The examiner started percussing a distance away from the organ and gradually approached it. The end point was reached when there was a sudden increase in loudness as the organ was approached. Subsequently, nearly all improvements in auscultatory percussion have been variations of the observations of Cammann and Clark, the later investigators using percussion in concentric circles, either toward or away from a stethoscope placed over the organ. Bianchi[7] in 1884 replaced the stethoscope with the phonendoscope, which had a rod with a small resonating chamber above it, and used scratching of the skin instead of percussion. In the ensuing years attention was centered in modifying the types of percussion used. Very light taps were used by some. Others used friction on the skin with the fingernails, going in concentric circles toward the organ.[8] Still others used friction strokes with the brush, moving away from a stethoscope placed on an organ.[9] Later, automatic hammers were used in conjunction with the stethoscope.

Most investigators placed great stock in their ability to outline the borders of the heart, abdominal masses, liver, and spleen with auscultatory percussion. Pal was able to diagnose two echinococcus cysts rather than one cyst in the liver of a patient.[8] The enthusiasm of these early workers knew no bounds; some even felt able to discern the place of the interventricular septum. Then came the inevitable reaction with the researches of de la Camp[10] and Sehrwald.[11] These authors, working on cadavers, showed that by placing the stethoscope at a certain spot and percussing toward it, they were able to shift the area of

the apparent left border of the heart at will. They concluded that certain friction patterns that were assumed to map out the size of the heart were really the result of conduction of the percussed patterns along ribs, sternum, muscle, and bone, and thus could be shifted at will if the stethoscope was moved.

More modern investigators[12, 13] seem to have overcome the objections of the early workers by using some object for direct percussion, and leading the sound thus elicited to the ears of the examiner. Auscultatory percussion with the resonant pleximeter falls into this class, although the instrument used was independently studied and does not resemble the other available instruments in any of its aspects.

COMMENT

The advantages of the instrument here discussed are:

1. It utilizes a direct percussion of the underlying structures and thus appears to do away with the disadvantage of the early methods.

2. The instrument can be easily procured at the present time and adapted for use.

SUMMARY

The instrument called the resonant pleximeter has been adapted for use in auscultatory percussion.

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Mr. Fleischer, of the Becton-Dickinson Company, cooperated in this study by supplying various types of pleximeters. The instrument used in this study can now be purchased in the market as the Fox localizing attachment.

177 Kingston Avenue

CLINICAL CHEMISTRY

THE INTRAVENOUS USE OF A SUCROSE-RINGER'S SOLUTION TO PRODUCE MAXIMAL DIURESIS*

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IN A PREVIOUS communication we reported on the determination of the relative merits of a series of solutions to produce diuresis without danger to the animal or injury to its kidney. The most consistent producer of diuresis which approximated the intravenous intake was 8 per cent sucrose in half strength Ringer's solution. When this was given for periods of six hours to each of eight rabbits at a rate of 100 c.c. per kilogram per hour, the animals all survived, and the average output was 88 per cent of the amount given in the six-hour period. At the time we reported that two of the animals had received multiple six-hour injections without serious difficulty and with a slightly greater diuresis in the later injections. Recently, Anderson and Bethea called attention to the marked changes in the convoluted tubules of the kidneys of persons who shortly before death had received sucrose solutions intravenously. It is undoubtedly true that these changes did not benefit the individual, but it is also true that they probably did him very little harm. After our work on both rabbits and dogs it seems reasonable to assume that the cell of the convoluted tubule can appear very much injured histologically and yet function normally, and can in a short time again return to normal appearance.

Our object in this study was to determine the innocuousness of repeated six-hour intravenous injections of sucrose-Ringer's solution when given at intervals of about a week. For this purpose we used three rabbits of about 2.5 kg., each animal receiving 100 c.c. per kilogram per hour for six hours. One animal received six, the second ten, the third fourteen, injections.

The first animal was killed three days after the sixth injection; the second animal died during the fourth hour of the eleventh injection, death being caused by the accidental injection of air; and the third died a traumatic death six days after its left kidney had been removed. In view of the changes occurring in the urine twenty-four to forty-eight hours after the first injection, two other animals received injections, one a single, and the other two.

In order to determine its value in other species of animals, the same solution was used in dogs in quantities of 50 c.c. per kilogram per hour for six hours.

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Three of the dogs received two injections only. The first dog received two injections on succeeding days; the second received the second injection forty-eight hours after the first; the third received the second injection one week after the first. The fourth dog, weighing 9.0 kg., received twenty-eight injections, twenty-seven before and one injection after removal of its left kidney. The following determinations were made routinely: blood urea, phenolsulfonphthalein output, output during six-hour diuresis, output during twenty-four-hour periods in the interval between injections, and specific gravity. Albumin, casts, erythrocytes, and leucocytes were tested for in the urine. A brief résumé of the experiments will show the trends without too much detail.

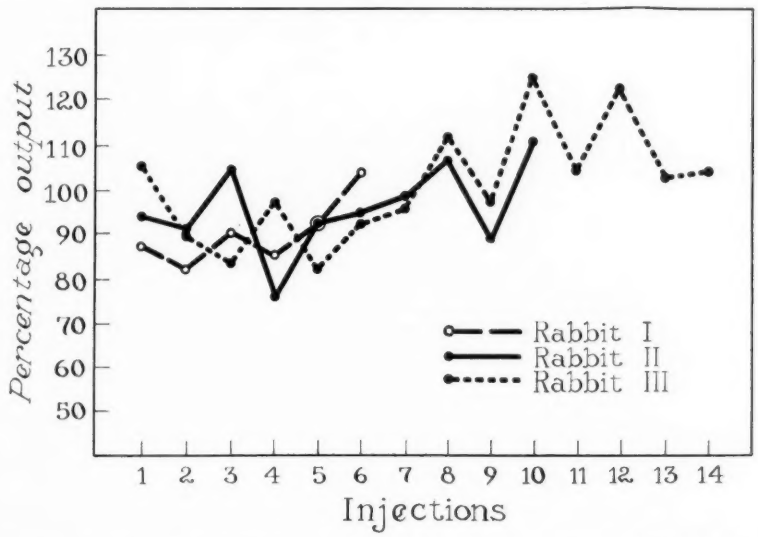

Fig. 1.—Output of urine after intravenous injection of sucrose-Ringer's solution as percentage of the amount injected.

Rabbit 1, weighing 2.3 kg., received six injections of 1,380 c.c. each of sucrose-Ringer's solution. The output varied from 1,150 to 1,440 c.c., or 83 to 104 per cent of the amount injected (Fig. 1); the average output was 90 per cent. The blood urea was 34 mg. per 100 c.c. before starting the injections, and at no time did it rise above 39 mg. The phenolsulfonphthalein output was 67 per cent before the start of injection and varied between 90 and 42 per cent. There was albumin, grade 3, in the urine in the second twelve-hour specimen after the first injection, and a trace on two occasions after that. Otherwise the urine was free of albumin. There were some erythrocytes and leucocytes in the specimen of urine taken forty-eight hours after the first injection; otherwise there were no formed elements. The animal was killed three days after the sixth injection. The findings at necropsy were essentially negative. Grossly, the kidneys were slightly enlarged and lighter in color than normal. Histologically, the convoluted tubules showed marked vacuolar degeneration and swelling of the cells, up to obliteration of the lumina of some of the tubules. The glomeruli looked compressed.

Rabbit 2, weighing 2.5 to 2.8 kg., was given ten injections of 1,620 c.c. each of sucrose-Ringer's solution. The output ranged from 1,235 to 1,810 c.c., or 76 to 112 per cent. Two of the last four injections produced diuresis of more than 100 per cent. The average output was 95.9 per cent of the amount injected. The blood urea ranged from 18 mg. per 100 c.c. to a high of 39 mg. after the fourth injection, with a return to 30 mg. at the time of death during the eleventh injection. The phenolsulfonphthalein output ranged from 60 to 97 per cent. There was albumin, grade 2, in the urine after the first injection only, with many casts after the first and seventh injections, and leucocytes after the first, second, seventh, and eighth injections. Fig. 1 (in the solid line) gives the percentage output of the various injections, showing the relatively better output in the later injections. The animal died and the kidneys were removed immediately and fixed in formalin. No cause of death other than air embolism was found, all the organs showing practically a normal state. The kidneys were not much enlarged.

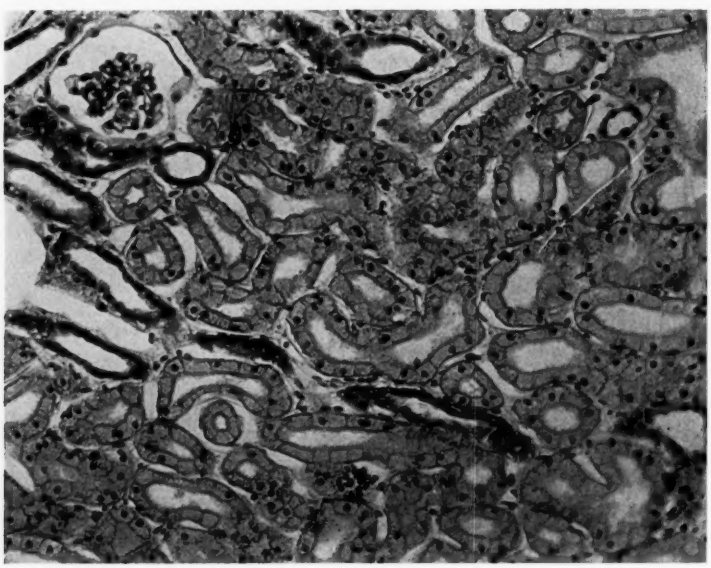

Fig. 2.—Separation of tubules in the kidney of a rabbit. The kidney was removed during the fourth hour of the eleventh injection of sucrose-Ringer's solution (×190).

They were brownish red and slightly lighter than the normal kidneys. The capsule was not adherent. The pelvis looked normal. On section the cortex was a little lighter in color than the medulla.

Microscopically, the striking feature was the uniformity of the cortex. All cells of the convoluted tubules were light pink, owing to staining of a finely granular protoplasm small in amount. The collecting tubules were lined by a low epithelium, which stained a uniform red in contrast to the pink convoluted tubules. Most of the convoluted tubules had a good-sized lumen. A few were almost swelled shut. Most of the nuclei were oval; some were pyknotic and shrunken. Some glomeruli seemed to have been distended; in others the outer wall of the capsule was fairly close to the glomerular tufts. In many areas there

was a considerable separation of the tubules as if by fluid (edema). Fig. 2 illustrates this condition of the kidney parenchyma.

Rabbit 3, weighing 2.3 to 2.9 kg., received fourteen injections of 1,560 c.c. each of sucrose-Ringer's solution. The output during the six-hour periods ranged from 1,285 c.c. to 1,890 c.c., an output of from 82 to 121 per cent. The average output in the six-hour period was 101 per cent; in seven injections the output was greater than 100 per cent, and in seven it was less than 100 per cent. The blood urea ranged from 14 to 30 mg. per 100 c.c. The maximal reading was 30 mg. per 100 c.c. after the third injection; the reading before the fourteenth injection was 18 mg. per 100 c.c. The single reading of the phenolsulfonphthalein output taken before the first injection was 67 per cent. All readings after this ranged between 82 and 96 per cent, except that twenty-four hours after the eleventh injection, which was given two days after the tenth injection, the phenolsulfonphthalein output dropped to 44 per cent. Five days later it was again 91 per cent. There was albumin, grade 1, in the urine twenty-four hours after the first injection and albumin, grade 4, after the second, but no albumin in the urine after any of the other injections. There were casts, grade 2, erythrocytes, grade 1, and leucocytes, grade 2, after the second injection, but they were absent after the other injections. Three days after the fourteenth injection the left kidney was removed. Six days later the animal died as a result of an injury sustained at the time of biopsy. Fig. 1 shows the percentage output of rabbit 3.

Tissue was removed from the rabbit three days after the fourteenth injection and again nine days after this injection. The specimens removed three days after the fourteenth injection were two small wedge-shaped pieces taken from the left kidney. The epithelium of the convoluted tubules was about normal in height and had lost its light, granular appearance. The protoplasm again stained a definite red and was collected toward the basement membrane. Strands extended out into the lumen, possibly the remains of the vacuolar degeneration. In some areas the vacuolar degeneration still remained more marked than in others. The glomeruli showed no change, and no mitotic progress was found in the convoluted epithelial cells.

The specimen removed shortly after death on the ninth day after the fourteenth injection showed a normal picture in the right kidney, but considerable change in the left, from which two wedges of tissue had been removed. In the left the convoluted epithelium still showed some vacuolar degeneration, and most of the tubules were filled with a pink, finely granular detritus. The right kidney could be very simply described as the picture of the normal rabbit kidney. The three photomicrographs show well the rapid return to normal of the epithelium after a marked functional change which gives the kidney the peculiar appearance seen in Fig. 2. Fig. 3 shows a condition of the convoluted epithelium after a period of three days, and Fig. 4, an almost complete return to normal after nine days.

In order to check still further the effects of the first and second injections, which seem to give the most marked reaction in the urine, two other rabbits were given injections, one a single, and the other two.

Rabbit 4, weighing 3.3 kg., put out 94 per cent of the 1,980 c.c. of sucrose-Ringer's solution injected in the first six-hour period and 84 per cent in the second. After the first injection the twenty-four-hour urine showed albumin, grade 4+, no casts, and a considerable number of leucocytes. The following twenty-four-hour specimen had a trace of albumin and no leucocytes. The phenolsulfonphthalein output dropped from 93 to 40 per cent in the twenty-four-hour period after the first, and from 87 to 55 per cent in the twenty-four hours after the second injection. The left kidney was removed twenty-four hours after the second injection. There was marked hydropic degeneration. The restitution to normal in the right kidney removed a week later was not nearly as complete as in the experiment in which only one injection was given.

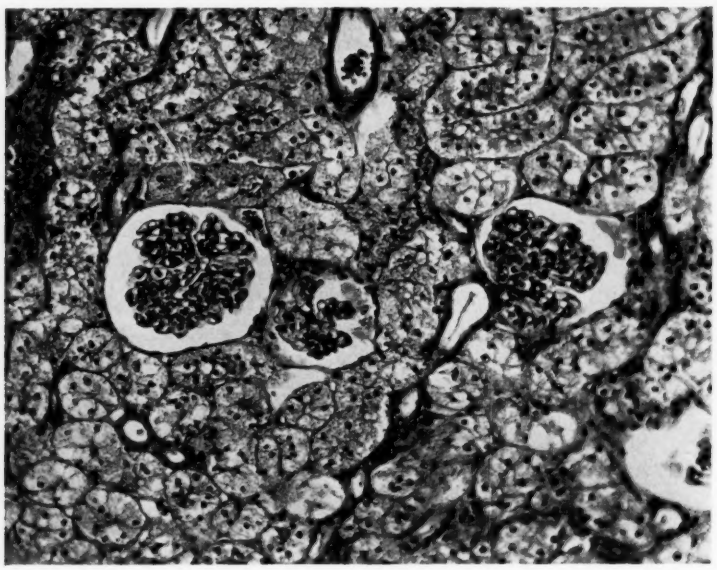

Fig. 3.—Appearance of the convoluted tubules in the kidney of a rabbit three days after the fourteenth injection of sucrose-Ringer's solution (×145).

Rabbit 5, weighing 2.2 kg., excreted 115 per cent of the 1,320 c.c. of sucrose-Ringer's solution intravenously injected in the six-hour period. In the twenty-four-hour period following the injection there were albumin, grade 4, erythrocytes, grade 1, and leucocytes, grade 1, but twenty-four hours later there was no albumin, erythrocytes, or leucocytes. The phenolsulfonphthalein output dropped from 75 to 49 per cent. The left kidney, removed twenty-four hours after the injection, showed marked hydropic degeneration and raggedness of cells. The right kidney was removed at necropsy one week later and showed practically a normal renal structure.

In order to determine what diuresis smaller quantities of 8 per cent sucrose in half strength Ringer's solution would produce, we gave two animals 50 c.c. of the solution per kilogram per hour for six hours, and two animals 25 c.c. per kilogram per hour.

Rabbit 6, weighing 2.1 kg., received 630 c.c. (50 c.c. per kilogram hour) and excreted 498 c.c. (38.5 c.c. per kilogram hour), or 79 per cent, in the six-hour

period. Rabbit 7, weighing 2.1 kg., received 630 c.c. (50 c.c. per kilogram hour) and excreted 446 c.c. (35.5 c.c. per kilogram hour), or 71 per cent, in the six-hour period. The average for the two rabbits was 75 per cent. Rabbit 6 was killed after twenty-four hours, and rabbit 7 after forty-eight hours. The changes were about as great as were those at a similar interval after injections of 100 c.c. per kilogram per hour for six hours. There was great vacuolar degeneration, irregularity of outline of the cells, and swelling of the cells to obliteration of the lumen of the tubules.

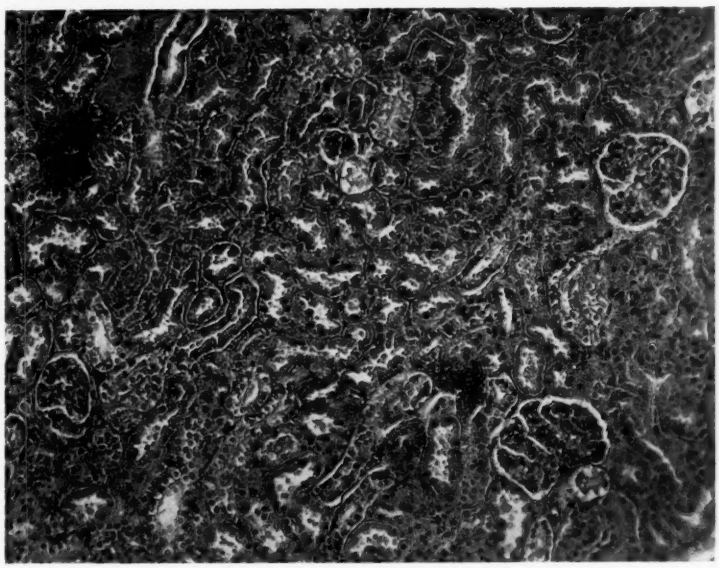

Fig. 4.—Return to normal of the convoluted tubules in the kidney of a rabbit nine days after the fourteenth injection of sucrose-Ringer's solution (×125).

The two animals that received only 25 c.c. per kilogram per hour for six hours excreted, one 31 per cent (8 c.c. per kilogram hour), and the other 50 per cent (12.5 c.c. per kilogram hour) of the amount injected, an average of 40 per cent, indicating the necessity of greater amounts of sucrose to produce diuresis. The histologic changes twenty-four and forty-eight hours after diuresis showed marked hydropic degeneration in the convoluted tubules. This was greater in the kidney removed after twenty-four hours than in the kidney removed after forty-eight hours.

Inasmuch as the rabbits that received 100 c.c. per kilogram per hour of the 8 per cent sucrose solution all survived, we thought it would be interesting to see the limit of the excretory capabilities of the rabbits' kidneys. Therefore, one animal was given 125 c.c. per kilogram per hour and another 150 c.c. per kilogram per hour. The animal receiving 125 c.c. per kilogram per hour put out 68 per cent (85 c.c. per kilogram hour) of the 2,172 c.c. injected, and showed no ill effect from the injection. The animal receiving 150 c.c. died forty-eight hours after the injection, having put out 88 per cent (132 c.c. per kilogram hour) of the 2,520 c.c. injected. Two other animals that received 150 c.c. per kilogram per hour for six hours died on the second day. One of the animals, which three

weeks before had received 125 c.c. per kilogram per hour, put out 92 per cent (138 c.c. per kilogram hour) of the 2,970 c.c. injected, and the other put out 88 per cent (132 c.c. per kilogram hour) of the 2,700 c.c. injected. It would seem that the limit that the rabbit can tolerate is between 125 and 150 c.c. per kilogram per hour. The average output for the three animals was 89 per cent of the amount injected (133.5 c.c. per kilogram hour), or 84 per cent of the body weight of the animal excreted in six hours, a very creditable performance.

In continuing our work with other animals, the dog seemed the one most easily available, and of such size that the total amounts injected would approximate amounts to be used for human injection.

Dog 1, weight 15.5 kg., received 50 c.c. per kilogram per hour for six hours on succeeding days. On the first day it received 4,620 c.c. in six hours and excreted 4,430 c.c. in the same time, an output of 96 per cent (47.6 c.c. per kilogram hour). The following day it received the same amount, but put out only 625 c.c., or 14 per cent (7 c.c. per kilogram hour), in the six-hour period. It gained 4 kg. this day, which it lost in the next three days. Its blood urea was 37 mg. per 100 c.c. before the first injection and 86 mg. before the second injection. The blood urea rose gradually to 233 mg. There were constantly many leucocytes in the urine and casts and erythrocytes after the first day. The phenolsulfonphthalein output fell to 10 per cent on the second day, but rose to 45 per cent the day before death. The animal died the fourth day after the first injection. At necropsy hemorrhages were found in the wall of the stomach and small and large bowel, as well as severe pyelitis with several huge abscesses in the kidneys. The epithelium showed marked vacuolar degeneration, but no necrosis of the epithelium.

Dog 2, weight 8.8 kg., received an intravenous injection of 2,640 c.c. in six hours and excreted 2,370 c.c. (45 c.c. per kilogram hour), or 90 per cent, during the first injection, and 1,875 c.c. (35.5 c.c. per kilogram hour), or 71 per cent, during the second injection the second day following. The animal appeared sick after the second injection and vomited two or three times daily. It died the fourth day after the first injection. Its phenolsulfonphthalein output was 80 per cent before the first injection, 65 per cent after the first, 65 per cent after the second injection, and 59 per cent two days after the second. There were albumin, grade 4, and erythrocytes, grade 4, after the first injection, as well as after the second injection. This animal also showed hemorrhages into the wall of the bowel and wall of the bladder. The kidney showed mild pyelitis, some sclerosis of the glomeruli, and marked changes in the convoluted tubules.

Dog 3, weight 12.6 kg., received two injections of 50 c.c. per kilogram per hour for six hours at an interval of one week. During the first injection it received 3,600 c.c. and excreted 3,000 (41.5 c.c. per kilogram hour), or 83 per cent, and at the second injection it received 3,780 c.c. and excreted 3,040 (40 c.c. per kilogram hour), or 80 per cent.

Dog 4, weight 9 kg., received twenty-eight injections of 50 c.c. per kilogram per hour for six hours. With one exception, when 2,675 c.c. was given, the animal received 2,790 c.c. during each six-hour period. The output varied from

1,830 c.c. during the first injection to 2,365 c.c. during the nineteenth injection, varying in output from 63 to 86 per cent of the amount injected, the average being 80 per cent (40 c.c. per kilogram hour) (Fig. 5). After the twenty-seventh injection the left kidney was removed. Five weeks later the output after injection of 2,790 c.c. was 2,100 c.c. (35.0 c.c. per kilogram per hour), or 75 per cent of the amount injected.

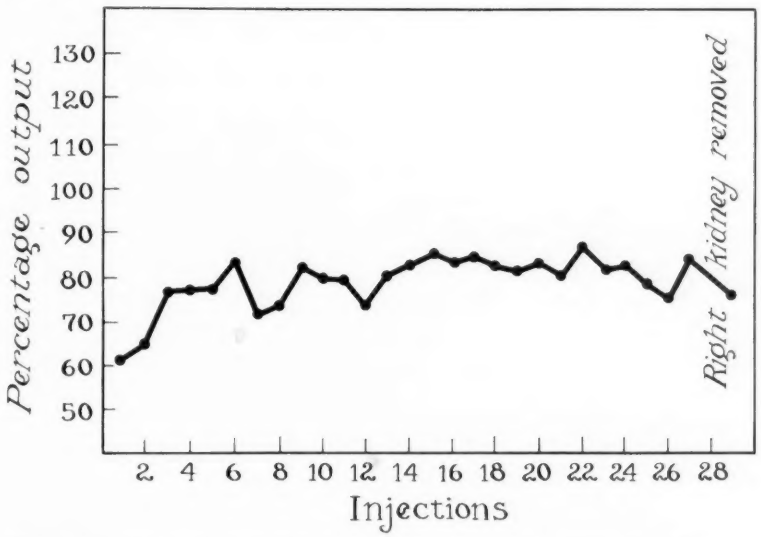

Fig. 5.—Output of urine after intravenous injection of sucrose-Ringer's solution as percentage of the amount injected.

The blood urea was 24 mg. per 100 c.c. before injection; it rose to 59 mg. after the first injection, went down to 33 mg. on the fourth day after the injection, rose again to 60 mg. after the second injection, and went down to 27 mg. on the second day. After this the range was between 18 and 38 mg. per 100 c.c., being 18 mg. and 20 mg. the day of the last two injections.

The phenolsulfonphthalein output was 80 per cent before the first injection; it fell to a trace at the end of twenty-four hours after injection, and was back to 63 per cent after forty-eight hours. It ranged between 58 and 94 per cent, with a reading of 26 per cent after the fifth injection and a trace twenty-four hours after the tenth and twelfth injections (given forty-eight hours after the ninth and eleventh injections).

There was albumin, grade 4, in the urine twenty-four hours after the first injection. The same was true after forty-eight hours. On the third day there was only a trace. Again after the tenth and eleventh injections there was albumin, grade 3, and after the twenty-fourth injection, albumin, grade 2.

Erythrocytes, grade 3, appeared after the first injection, and erythrocytes, grade 1, after the fourth, sixth, and seventh injections. Leucocytes in numbers were present after the first and eleventh injections, and a few after twenty-one injections. Constant catheterization probably accounted for these few cells. Nothing unusual occurred after the twenty-eighth injection, which was given to the dog after removal of the left kidney.

In these dogs as well as in the rabbits, the first injection seems to give rise to the most marked changes in the urinary findings and the phenolsulfonphthalein and urea values. It will be noted that the tenth, twelfth, and twenty-fourth injections were given forty-eight hours after the ninth, eleventh, and twenty-third injections, instead of the usual weekly interval, and this may account for the changes after these injections. On the other hand, it can be said that the twenty-fifth and twenty-seventh injections were given at two-day intervals without any change in the phenolsulfonphthalein output, rise in blood urea, or erythrocytes in the urine.

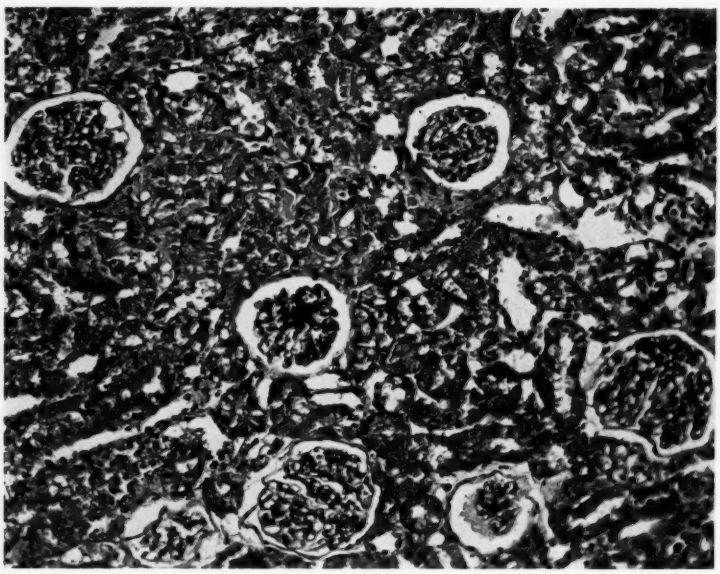

Fig. 6.—Condition of the kidney of Dog 4 five days after the twenty-seventh injection of sucrose-Ringer's solution (×125)

It is, of course, difficult to say why marked changes are observed after one injection and not after another of the same character. The changes observed were definitely temporary, and even repetition of the same insult produces neither the same acute change nor any permanent damage in the histologic structure or the function of the kidney.

The left kidney of the dog, removed five days after the twenty-seventh injection, showed no changes from the appearance of a normal dog's kidney (Fig. 6). This, together with the fact that the remaining kidney five weeks after left nephrectomy could excrete 75 per cent of the amount injected, shows excellent remaining functional capacity of the animal, whose two kidneys averaged 80 per cent in twenty-seven injections.

COMMENT

It would seem that in attempting to find a solution that, when given in large amounts intravenously, produces an equally large diuresis, the first requisite must be safety. Does the solution suggested have this requisite? From the number of injections given, we feel that we can safely say that the epithe-

lium, though markedly changed histologically, seems able to function in the changed condition. There is the second objection that more frequently the most severe reaction comes after the first injection with casts, albumin, and sometimes erythrocytes in the urine. This is, however, only a temporary reaction, and occurs only very occasionally after the later injections. Our object in this experiment is to determine the maximal excreting power of the kidney, so as to remain well within the limit of safety when applying these results to treatment in man.

It seems to us that after the results here obtained, it would be justifiable to use this solution clinically in cases in which there was an emergency in which it was necessary to produce rapid washing out of the urinary passages, as in acute pyelonephritis, to relieve obstruction with or without infection and high blood urea, to prevent ascending infection after operation on the prostate or other lower urinary obstruction, and also to produce good diuresis after a plastic operation on a hydronephrotic kidney.

SUMMARY

The rabbit can excrete from 90 to 100 per cent of fluid, representing from 54 to 60 per cent of its body weight, when given in the form of 8 per cent sucrose in half strength Ringer's solution at a rate of 100 c.c. per kilogram per hour for a six-hour period. Such injections may be repeated as often as every other day, with equally effective diuretic effect and no detectable impairment of renal function. A rabbit can excrete 89 per cent (133 c.c. per kilogram hour) of the injected fluid when given at a rate of 150 c.c. per kilogram per hour, a total of 84 per cent of its body weight in six hours, but most of these animals die within forty-eight hours after injection.

The dog can excrete 80 per cent of the fluid given intravenously in the form of 8 per cent sucrose in half strength Ringer's solution when given at a rate of 50 c.c. per kilogram per hour for a six-hour period weekly.

The kidneys in neither animal show any permanent damage after multiple injections, and regain their normal histologic structure in a period of ten days.

The first injection is the one after which the functional capacity of the kidney seems most likely to be lowered, but this lowering is only temporary.

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VARIATIONS OF SERUM MAGNESIUM IN 52 NORMAL AND 440 PATHOLOGIC PATIENTS*

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CONSIDERABLE data are being published relative to the variations of serum magnesium in health and disease. Upon examination of this information, it is apparent that too little is known at the present time to report with finality that certain abnormalities seen in man are due to a derangement of magnesium metabolism. A definite tendency toward a hypomagnesemia or hypermagnesemia seems to exist in certain clinical entities, and only after the accumulation of considerable data will it be possible to ascertain whether or not a disturbance in magnesium metabolism may play a role in the etiology of disease. Hence this investigation was undertaken.

EXPERIMENTAL

Serum magnesium determinations were made on 52 normal and 440 pathologic persons. The normal subjects were medical students and laboratory technicians. The pathologic cases were all hospitalized patients at the time that the blood samples were taken and analyzed. Since ingestion of food has no particular influence on the blood magnesium, no attempt was made to obtain the samples of blood at any particular time in reference to meals. The majority of the determinations were made by the modification of the titan yellow method,[1] yet a few were made by the method described by Briggs.[2]

As can be seen from Table I, the serum magnesium values in the majority of the normal persons (79 per cent) range between 2 and 3 mg. per 100 c.c. The average serum magnesium for the entire group is 2.48 mg. per 100 c.c. This corresponds very closely to the average mean of 2.35 mg. per 100 c.c. of serum, as reported by 17 different investigators (see Table II). In our group only one normal person was found to have a magnesium value slightly below 1.7 mg. We consider, therefore, 1.7 and 3.1 mg. of magnesium per 100 c.c. of serum as the lower and upper limits of normal, respectively.

Nothing unusual is noted in the values obtained from those patients suffering from endocrine disturbances. Our results on diabetic patients support the variability of blood magnesium as already noted by other investigators. In 12.5 per cent of the patients suffering from diabetes mellitus we found a low serum magnesium. This was previously reported by Blumgarten and Rohdenburg.[19] In 15 (26 per cent) of our cases a hypermagnesemia occurred. This confirms the findings of Watchorn and McCance.[5]

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The few patients suffering from toxic thyroid gland and polyglandular disturbances, which we have examined, show that a low blood magnesium may prevail. Blumgarten and Rohdenberg[19] report that 15 of their 45 patients suffering from goiter had a low blood magnesium. Recently, Soffer and coworkers[20] made the interesting observation that the ultrafiltrable magnesium in 31 patients with hyperthyroidism was low (average 64 per cent) when compared to that found in 14 normal persons (average 85.5 per cent).

More definite findings have been obtained in patients suffering from renal and circulatory diseases. The majority of the patients with chronic nephritis, as seen in Table I, reveal an elevated serum magnesium (average 3.56 mg. per 100 c.c.). Most of these patients also had an impairment of kidney function. It is interesting to note that those patients having hypertension associated with definite renal impairment also had an elevated serum magnesium (average 3.90 mg. per 100 c.c.). Hypertensive and arteriosclerotic patients, without apparent renal damage, had a definite though less marked elevation of blood magnesium. This would seem to bear out the inference of Becher and Hamann[21] that the concentration of this ion is increased in hypertension as a result of the associated kidney damage. Numerous reports have appeared in the literature which would indicate that a retention of magnesium is rather common in cases of renal insufficiency.[5, 9, 10, 14, 21-25]

Those patients suffering from vasospastic diseases present a different type of picture. More than 17 per cent of the group have a hypomagnesemia. The group as a whole is somewhat below normal in so far as the mean blood magnesium is concerned (2.12 mg. per 100 c.c. of serum). From these figures it would appear that some relationship may exist between hypomagnesemia and spasticity of the smooth muscles in the blood vessels.

From Table I it may be noted that 23 per cent of the 26 patients suffering from malignant neoplasms have a low serum magnesium. Other investigators[19, 26, 27] have reported similar findings. This ion has on occasion been implicated as one of the etiologic factors in the cause of cancer.[28]

In the patients suffering from hepatic disorders, especially those associated with jaundice, a significant increase in the serum magnesium is noted. Becher and Hamann,[21] Blumgarten and Rohdenburg,[19] and Jung[29] have reported similar findings. In this connection it is interesting to note that Rothman, Meranze, and Meranze[30] found the blood phosphatase to be high in cases of obstructive jaundice. Erdtmann[31] had previously recorded that phosphatase activity is greatly increased by the addition of magnesium.

Far too few cases of infectious diseases have been studied to draw any conclusions from the results. In our patients 3 of the 5 cases of chronic arthritis studied had a low serum magnesium.

The blood of 8 women suffering from toxemia of pregnancy was studied. It seems significant that 3 of these should have a low serum magnesium. One of these patients who had a magnesium concentration below 1 mg. per 100 c.c. of serum also had severe convulsions. Probably the successful use of magnesium sulfate in the treatment of eclampsia[32-35] depends, at least in some cases, upon the elevation of the blood magnesium to the proper physiologic level.

TABLE

DETAILED ANALYSIS OF THE SERUM MAGNESIUM

The bold face vertical lines represent the

CLINICAL DIAGNOSIS	NO. OF PATIENTS	MILLIGRAMS OF MAGNESIUM								
		BELOW 1.0	1.0 TO 1.2	1.2 TO 1.4	1.4 TO 1.6	1.6 TO 1.7	1.7 TO 1.9	1.9 TO 2.1	2.1 TO 2.3	2.3 TO 2.5
Normal individuals	52	–	–	–	–	1	3	3	8	15
1. Endocrine disorders:										
Diabetes mellitus	56	2	–	3	1	1	1	2	3	2
Toxic adenoma of thyroid	10	1	–	–	1	1	1	–	1	1
Polyglandular disturbances	5	–	–	–	1	1	1	–	–	–
2. Circulatory disorders:										
Arteriosclerosis	15	–	–	–	–	–	–	–	–	1
Hypertension without renal functional impairment	65	1	–	–	–	1	–	–	3	5
Hypertension with renal functional impairment	23	–	–	–	–	–	–	–	–	–
Vasospastic diseases	52	–	–	1	2	6	11	9	10	3
3. Renal disorders:										
Chronic nephritis	26	–	–	–	–	1	–	–	–	–
Acute nephritis	3	–	–	–	–	–	1	–	1	–
Renal calculi	3	–	–	–	–	–	–	–	–	–
Urinary infections	5	–	–	–	–	–	–	–	–	–
Miscellaneous	5	–	–	–	–	1	–	–	1	–
4. Pulmonary disorders:										
Chronic pulmonary infections (including tuberculosis and pleural infections)	14	1	–	–	–	1	1	1	–	1
Pneumonia (lobar)	5	–	–	–	–	–	–	1	1	–
5. Neoplastic disorders:										
Malignant neoplasms	26	–	–	3	1	2	2	2	2	–
Benign neoplasms	5	–	–	–	–	–	–	1	1	1
Prostatic hyperplasia	15	–	–	–	–	–	2	1	2	2
6. Hepatic disorders:										
Chronic calculus cholecystitis	16	1	–	–	2	1	–	1	–	2
Chronic noncalculus cholecystitis	13	–	–	–	–	–	2	1	1	1
Acute catarrhal jaundice	6	–	–	–	–	–	–	–	–	–
Cirrhosis of liver	9	–	–	1	–	–	1	2	–	1
7. Infectious diseases:										
Acute rheumatic fever	3	–	–	–	–	–	–	–	–	–
Chronic arthritis	5	1	–	–	2	–	–	1	–	1
Mastoiditis and otitis media	3	–	–	–	–	–	–	–	–	–
Diseases of the eye	4	–	–	–	–	–	–	1	–	–
Superficial abscesses	3	–	1	–	–	–	–	–	–	–
8. Obstetric:										
Normal pregnancies	4	–	–	–	–	1	–	2	–	1
Toxemia of pregnancy	8	1	2	–	–	–	–	–	–	–
Gynecologic diseases	7	–	–	–	–	–	2	2	–	–
9. Diseases of the central nervous system	7	–	–	–	–	–	1	1	–	1
10. Orthopedic surgical problems	6	2*	–	–	–	1	1	–	–	–
11. Epilepsy	5	–	1	–	1	–	–	1	1	1
12. Peptic ulcers	8	–	–	–	–	–	–	–	1	1

*Both were cases of ununited fractures.

†Intra-articular adhesions of right shoulder with beginning ossification of the joint.

I

VALUES IN NORMAL AND PATHOLOGIC PATIENTS

lower and upper limits of the normal range.

PER 100 C.C. OF SERUM													
2.5 TO 2.7	2.7 TO 2.9	2.9 TO 3.1	3.1 TO 3.3	3.3 TO 3.5	3.5 TO 3.7	3.7 TO 3.9	3.9 TO 4.1	4.1 TO 4.5	4.5 TO 5.0	5.0 TO 6.0	6.0 TO 7.0	7.0 TO 8.0	MEAN
13	5	3	1	–	–	–	–	–	–	–	–	–	2.48
5	12	9	8	3	3	1	–	–	–	–	–	–	2.69
2	1	1	1	–	–	–	–	–	–	–	–	–	2.51
1	1	–	–	–	–	–	–	–	–	–	–	–	2.07
3	6	2	2	–	–	–	–	1	–	–	–	–	3.05
8	15	16	9	1	2	2	1	1	–	–	–	–	2.88
1	2	5	1	1	–	1	5	3	1	2	–	1	3.90
3	4	2	1	–	–	–	–	–	–	–	–	–	2.12
4	1	3	3	–	2	6	2	2	1	1	–	–	3.56
1	–	–	–	–	–	–	–	–	–	–	–	–	2.31
–	1	1	1	–	–	–	–	–	–	–	–	–	3.00
–	2	2	–	1	–	–	–	–	–	–	–	–	2.97
1	1	1	–	–	–	–	–	–	–	–	–	–	2.50
1	2	1	1	1	2	1	–	–	–	–	–	–	2.66
2	–	–	1	–	–	–	–	–	–	–	–	–	2.49
1	4	2	5	1	–	1	–	–	–	–	–	–	2.10
–	1	–	1	–	–	–	–	–	–	–	–	–	2.51
2	1	3	–	1	1	–	–	–	–	–	–	–	2.61
–	2	1	–	2	1	1	–	2	–	–	–	–	2.73
–	2	3	2	–	–	–	1	–	–	–	–	–	2.73
–	–	2	–	1	1	1	–	–	1	–	–	–	3.56
–	–	1	1	1	1	–	–	–	–	–	–	–	2.48
–	1	–	–	1	1	–	–	–	–	–	–	–	3.33
–	–	–	–	–	–	–	–	–	–	–	–	–	1.71
–	1	1	–	–	1	–	–	–	–	–	–	–	3.16
2	–	1	–	–	–	–	–	–	–	–	–	–	2.52
–	1	1	–	–	–	–	–	–	–	–	–	–	2.31
–	–	–	–	–	–	–	–	–	–	–	–	–	2.28
–	1	1	2	–	–	1	–	–	–	–	–	–	2.38
–	1	1	–	1	–	–	–	–	–	–	–	–	2.45
–	2	1	–	–	–	–	–	–	–	–	–	–	2.68
–	–	–	–	1	–	–	–	1†	–	–	–	–	2.17
–	–	–	–	–	–	–	–	–	–	–	–	–	1.85
2	1	1	–	–	1	1	–	–	–	–	–	–	2.80

We studied 3 cases with orthopedic surgical conditions. In 2 of these a marked hypomagnesemia and in one a marked hypermagnesemia occurred. The 2 patients with hypomagnesemia were cases of ununited fractures of the patella and tibia, and their serum magnesium values were 0.96 and 0.80 mg. per 100 c.c. of serum, respectively. Further study revealed that the latter patient required an amputation of the limb one year after the above blood study was made. The patient with a hypermagnesemia of 4.4 mg. per 100 c.c. of serum suffered from intra-articular adhesions of the right shoulder with beginning ossification of the joint.

TABLE II

CONCENTRATION OF SERUM MAGNESIUM IN NORMAL PERSONS

AUTHORS	PRECIPITATING AGENT	METHOD	RANGE OF VALUE	MEAN
Briggs[2]	Phosphate	Colorimetric	2.23-2.50	
Kramer and Tisdall[3]	Phosphate	Colorimetric	1.80-2.30	2.10
Bogert and Plass[4]	Phosphate	Colorimetric	1.90-2.70	2.30
Watchorn and McCance[5]	Phosphate	Colorimetric	2.30-2.66	2.48
Becher[6]	Phosphate	Colorimetric	1.80-2.30	
Wacker and Fahrig[7]	Phosphate	Colorimetric	2.00-2.97	2.40
Cope[8]	Phosphate	Colorimetric	1.82-2.63	2.06
Walker and Walker[9]	Phosphate	Colorimetric	1.60-3.00	2.20
Brookfield[10]	Phosphate	Colorimetric	1.89-2.19	2.04
Bomskov[11]	Hydroxyquinoline	Bromination	1.70-2.60	
Greenberg and co-workers[12]	Hydroxyquinoline	Bromination	2.00-3.66	2.74
Velluz and Velluz[13]	Hydroxyquinoline	Bromination	1.60-2.40	2.00
Raices[14]	Hydroxyquinoline	Bromination	1.69-3.00	2.44
Hoffman[15]	Hydroxyquinoline	Colorimetric	1.90-2.50	2.18
Hirschfelder and Haury[16]	Titan yellow	Colorimetric	1.80-2.40	2.11
Haury[17]	Titan yellow	Colorimetric	1.70-3.10	2.33
Wolf[18]*	Titan yellow	Colorimetric	2.90-4.00	3.61

*For whole blood.

The 3 cases suggest to us the possibility that a deficiency of magnesium may be partly responsible for the delayed formation of callus. Conversely, a hypermagnesemia may play some etiologic role in the production of intra-articular adhesion and ossification.

In epilepsy a deficiency of magnesium was found in 2 of the 5 patients examined. This is in close agreement with the findings of Denis and Talbot,[36] Blumgarten and Rohdenburg,[19] Wolf,[37] and Hirschfelder and Haury.[38] This is not surprising since the establishment of the close relationship between magnesium deficiency and convulsions.[39-43] However, Greenberg and Aird[44] found the blood serum magnesium essentially normal in 22 patients with essential epilepsy.

SUMMARY

The results of serum magnesium determinations made in our laboratory on 52 normal and 440 pathologic patients are reported and the results are briefly discussed.

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THE ABSORPTION OF STILBESTROL AND THEELIN FROM CYSTS OF SESAME AND PEANUT OILS*

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A LARGE number of animals treated in various ways with stilbestrol were autopsied during the last two years. Cysts were always found in the rats given oil by intramuscular injections and usually found after subcutaneous injections. The estrogenic content of the oil from many of these cysts was studied.

METHODS

The procedure followed was an outgrowth from experiments designed to test the minimal dose of stilbestrol. Failure to obtain consistent results with stilbestrol in oil led to a more careful study of the absorption of the oil. Data were taken from a total of 260 albino rats. Forty female rats used for minimal dose testing formed the beginning of these experiments. This group was given 0.3 c.c. of sesame oil in three doses of 0.1 c.c. each, 20 being injected intramuscularly and 20 in the subcutaneous tissue of the flank. A second group of 30 castrated mature female rats were injected subcutaneously in the back and 90 male rats intramuscularly in the hind legs with 1.0 mg. of stilbestrol in 1.0 c.c. of sesame oil. For the purpose of comparing theelin with stilbestrol, an additional 60 female rats were injected intramuscularly with 1.0 c.c. of either peanut oil or sesame oil containing 1.0 mg. of stilbestrol or theelin, and estrous cycles were followed for a period of thirty days (Fig. 4). Forty males injected intramuscularly with theelin were also added for cyst studies (Fig. 3). The needle was inserted to a depth of about 2 cm., and without further manipulation the 1.0 c.c. of the oil was deposited in one globule. The skin was pinched to

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prevent leakage of the oil. Some of these rats were autopsied each day for a period of forty days, and thereafter at various times up to a period of nine months after the injections were made. The oil from the cysts of each leg was collected separately, shaken in 5.0 c.c. of a salt solution (0.9 per cent), and the resulting oil emulsion given intraperitoneally to a castrated female rat. The estrous cycles were taken twice daily by the pipette method[1] as long as the rats remained in estrus.

RESULTS

The 40 rats that were given doses of 0.1 c.c. of sesame oil all showed multiple cyst formation in the muscles and the subcutaneous tissue of the flank. These cysts were usually small, 1 to 3 mm. in diameter, and full of clear oil.

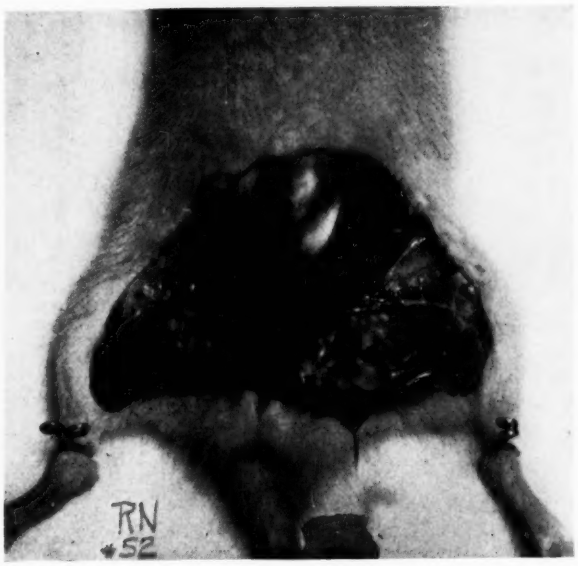

Fig. 1.—A castrated female rat used for minimal dose testing of stilbestrol and given several subcutaneous injections of sesame oil in amounts of 0.1 c.c. each. The cysts of oil are typical of rats injected with sesame oil.

The cyst wall was firm and could easily be dissected free (Figs. 1 and 2). The quantity of stilbestrol injected was so small that tests for its estrogenic content from the encysted oil were not made. Only occasionally were large cysts present in these rats. Likewise the 30 rats injected with 1.0 c.c. of oil subcutaneously in the back usually showed similar cysts, but these were less abundant, indicating that only part of the oil as it gravitated downward from the back had remained in one area long enough to become encysted. At autopsy the 90 male rats which were injected intramuscularly with 1.0 c.c. of sesame oil showed larger cysts than the previously mentioned groups, and from these sufficient oil for testing could be obtained. The amount of oil collected from the cysts in the leg varied from 0.2 to 1.0 c.c. and diminished somewhat as postinjection time increased (Fig. 3). The cyst wall was found as early as the third day after the injections were made, and after five or six days it was always present. Up to at least a month after the cysts were formed, the oil was not always clear,

being frequently creamy to cheesy in consistency, and white in color. Although observations on cysts of longer duration are less in number, they indicate that the oil may be entirely clear, or clear with white, cheesy masses floating in it for at least nine months after treatment.

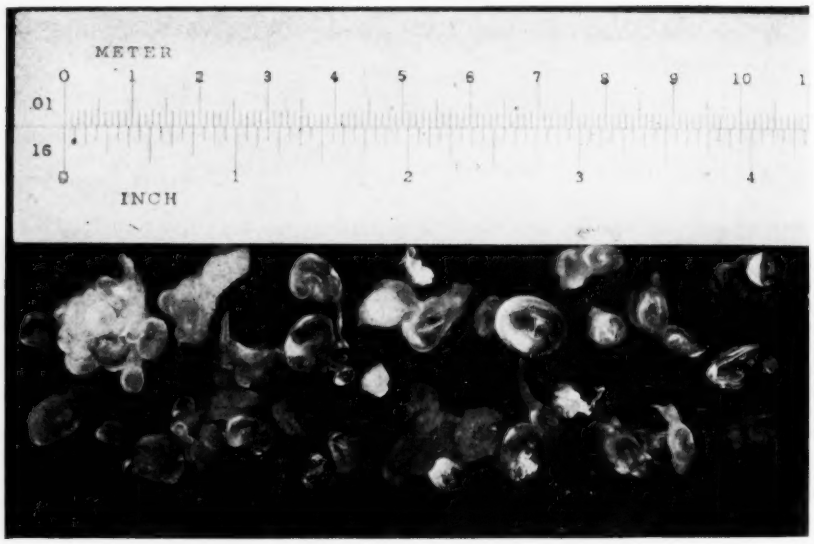

Fig. 2.—Cysts containing sesame oil dissected after being in the subcutaneous tissue and muscles of rats for varying periods up to nine months.

Fig. 3 depicts the activity of the stilbestrol remaining in the cystic oil after one to thirty-six days in the muscles. This curve, as first constructed from 60 tests, was very irregular. Since this might be expected from scattered data, an additional 30 tests were made at the irregular points. Therefore, it is fairly regular and shows that the percentage of rats giving positive vaginal smears diminish from 82 per cent on the first to third day to zero percentage at twenty-three days. One animal did not react when given 0.4 c.c. of cyst oil on the third day. This is difficult to evaluate, but since the slope of the curve is downward at four to six days and continues downward thereafter, these negative cases are not incidental to the technique of testing but rather indicate that stilbestrol was not present in sufficient amounts to give a positive vaginal reaction. Only one positive test was obtained after the twenty-first day, and this was from oil after twenty-nine days in the muscle (Fig. 3).

In a similar manner, 40 males were injected with peanut oil containing theelin to determine the theelin remaining in the cysts. Peanut oil, like sesame oil, became readily encysted. Theelin disappeared from these oil cysts sooner than the stilbestrol, the curve dropping sharply from the second to the eighth day. After the eleventh day further positive reactions were not found (Fig. 3). The difference between the amounts of stilbestrol and theelin remaining in the cystic oil may be explained by solubility. The theelin being more soluble in water was more readily removed by the tissue fluids surrounding the cysts, whereas the stilbestrol, although very soluble in oil, was insoluble in water.

The duration of estrus after a single injection of 1.0 c.c. of peanut oil containing 1.0 mg. of stilbestrol or theelin was determined in adult female rats. Fig. 4 shows the percentage of rats in estrus plotted against time in days. It is clearly seen that the curve for the stilbestrol-treated rats dropped slowly from the eighth to the twenty-first day, whereas the comparable curve for theelin absorption was considerably below this, having started downward

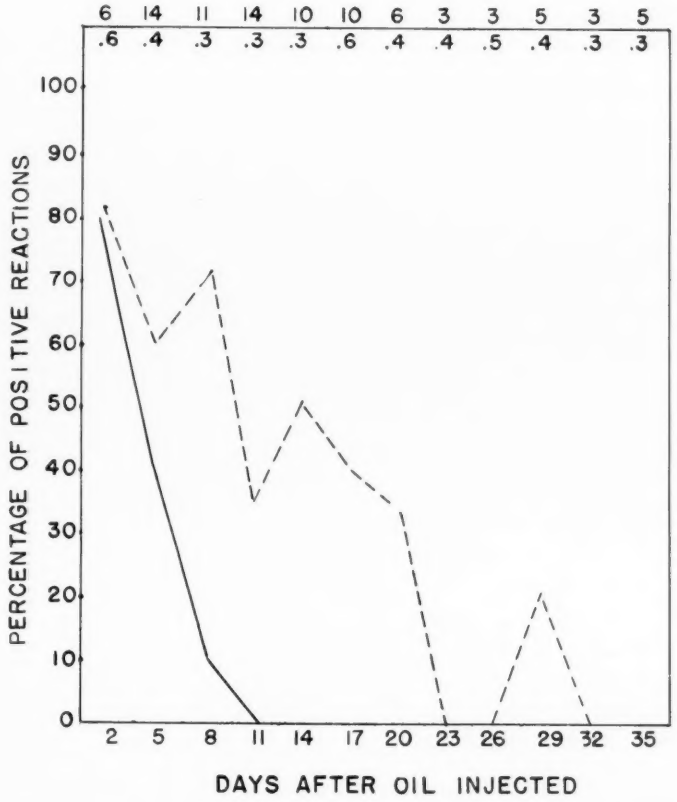

Fig. 3.—The per cent of castrated rats showing estrus after a single injection of oil from cysts containing stilbestrol (upper curve, 90 rats) and theelin (lower curve, 40 rats). Abscissa (lower), time in days that the oil was in the muscles of the donor rats. Abscissa (upper, below line), mean amounts of oil in cubic centimeters recovered each three-day period from the original 1.0 c.c. of sesame oil injected in the muscles of the donor rats. These figures also represent the amount of oil reinjected into the 90 test rats forming the upper curve. Abscissa (upper, above line), the 90 rats used for the stilbestrol curve subdivided to show the number of tests made at three-day intervals. The 90 male rats received 1 c.c. of sesame oil containing 1.0 mg. of stilbestrol. The 40 males received 1 c.c. of peanut oil containing 1.0 mg. of theelin. Both male groups were treated once only; the 1 c.c. of oil being injected in one globule in the muscles.

sharply on the fifth day and reached zero by the fourteenth day, thus indicating the more rapid absorption of theelin. These curves also conform in time, and to a large extent attest the validity of the estrous curves obtained with the oil from the cysts (compare Fig. 3 with Fig. 4). For the purpose of comparison the absorption of stilbestrol from both peanut oil and sesame oil is shown in Fig. 4. The two curves are identical until the ninth day and are only slightly separated thereafter, as indicated by the dotted line in Fig. 4.

DISCUSSION

The multiple cysts found in the subcutaneous tissue and muscles of rats given minimal doses of stilbestrol in a total of only 0.3 c.c. of sesame oil (Fig. 1) show that oil itself is poorly absorbed and leads to lack of confidence that stilbestrol could be uniformly absorbed through the fairly thick walls of the cysts. These oil pockets were found to be encysted by the fifth day, and the cyst wall appeared to become thicker and more durable for some time thereafter. Further evidence that sesame or peanut oil is an inadequate vehicle for stilbestrol was also obtained with larger doses of stilbestrol because they show that in some cases

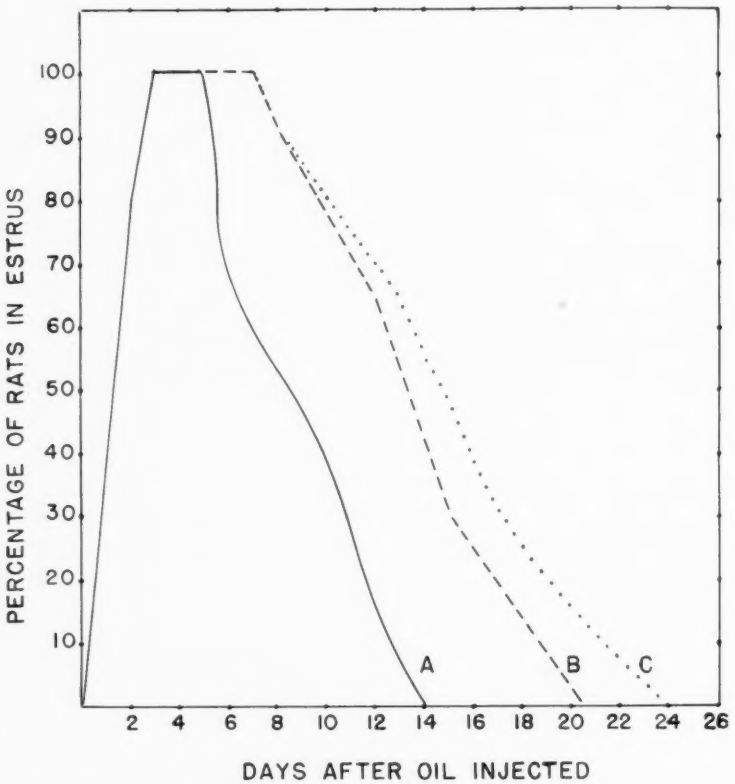

Fig. 4.—Lower curve, *A*, the per cent of rats in estrus each day after 1.0 mg. of theelin in peanut oil was given intramuscularly. Upper curve, *B*, after 1.0 mg. of stilbestrol in 1.0 c.c. of peanut oil was injected intramuscularly. Dotted line, *C*, same in sesame oil. Twenty castrated rats were used in each of the three groups.

all the stilbestrol (1.0 mg.) may be absorbed in three days, while in other rats given identical treatment, the stilbestrol was still present after being in the muscles for twenty days (Fig. 3). Such a range of time for complete absorption probably would be less for smaller doses; but the uncertainty as to when all the stilbestrol would be absorbed makes minimal dose testing with oil solutions confusing.

After more than 150 tests were carefully studied, hope of determining the minimal dose of crystalline stilbestrol in oil was abandoned. Much confusion still exists in the literature concerning oil and aqueous solutions of estrogens. Korenchevsky, Dennison, and Schalit[2] found that oil remained several days after

intramuscular injections and discussed the uncertainty of oil absorption. Even more impressing data were obtained by Deanesly and Parkes,[3] who found estrin in castor oil after fifteen days in the subcutaneous tissues of mice, and suggested that oil is a highly unsuitable medium for quantitative assay. On the other hand, Bülbring and Burn,[4] using the weight of the uterus as an index of estrone activity, felt that their error in testing was small. The sensitivity of the uterus to small amounts of estrone in the blood[5, 6] and the duration of the uterine growth test, which is usually beyond the time required for vaginal smear readings, give evidence that this test may be quite reliable for oil solutions. Also, estrogens in oil solutions deteriorate less readily than in aqueous solutions.[7] These observations extended by these data led to the conclusion that oil was a vehicle from which absorption not only was slow and variable but also had the disadvantage of almost always becoming encapsulated and remaining in a cystic state for many months (up to nine months, Fig. 2; for as long as fifteen months not illustrated).

Oils differ in their ability to form cysts. Castor oil produced more cysts than sesame oil, and the latter produced more than olive oil.[3] The present experiments indicate a similarity between sesame oil and peanut oil, judging by their ability to form cysts and by the duration of estrus induced by the stilbestrol remaining in these oil cysts (Fig. 4). Yet the wall of the sesame oil cysts appeared thicker than the wall of the cysts usually obtained with peanut oil.

SUMMARY

The formation of cysts following injections of sesame oil and peanut oil was observed in all rats (210) injected intramuscularly and was usually present in rats (50) injected subcutaneously. Oil obtained from the cysts was tested on castrated rats for stilbestrol and theelin. The curves for these tests showed that in some cases all the estrogenic activity was gone by the third day, but in a few rats some stilbestrol remained in the cysts even after twenty days. This is in marked contrast to theelin, in which case the time of retention was reduced to approximately nine days. These cysts, containing oil, remained in the tissues for many months.

Castrated rats were given 1.0 mg. of stilbestrol or theelin in 1.0 c.c. of peanut oil, and the estrous cycles were taken daily. These also confirmed the slow absorption of stilbestrol compared with theelin after a single oil injection.

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HISTAMINASE

Further Laboratory Studies

Maurice Vaisberg, M.D., Baldwin, N. Y.

HISTAMINASE is the term applied to the histamine-inactivating property of aqueous extracts of certain animal body tissues. The properties and actions of histaminase closely resemble those of the enzymes, thus tentatively placing this "substance" in the category of enzymes or ferments. The property was first described by Eustis,[1] who reported inactivation of histamine with the pulp from the liver of a turkey buzzard. Most of the original research upon the characteristics of histaminase is described in the excellent papers of Best,[2] Best and McHenry,[3] and McHenry and Gavin.[4-7] Their exhaustive research has been the basic and guiding principle in this work and in previous work I have done. In the previous publication[8] there was described the method of preparation, crude standardization, titration, and some clinical application of histaminase.

Method of Preparation and Titration

As previously described,[8] hog kidney, after mincing, was defatted with acetone, dried with ether, and finely ground. The powder was extracted with phosphate buffer at pH 7.2 under toluene overnight in the incubator at 37° C. All the titrations and tests were performed with my method on my own left forearm, as previously described (except the experiments with trypsin). However, by further refinement, it was possible to arrive at an almost exact determination of the potency. The method follows: The sensitivity of the skin to histamine was first determined. One-tenth cubic centimeter of increasing dilutions of histamine phosphate was injected intracutaneously, and the size of the resulting wheals was measured and recorded.

CONCENTRATION	WHEALS IN MM.
1:10,000	18
1:100,000	17
1:1,000,000	15
1:10,000,000	12
1:100,000,000	9

Hence one could consistently determine one part in five million, or 0.05 gamma of histamine phosphate injected. Thus the skin is less sensitive than the hen's ileum,[9] which can detect 0.01 gamma per cubic centimeter. In actual titration small Florence flasks were used. Into each flask measured amounts of histaminase solution in decreasing quantities were placed, e.g., 1 c.c. solution, 0.5 c.c. solution, 0.25 c.c. solution, etc. To each flask was then added 0.25 mg., or 0.25 c.c., of 1.1,000 histamine phosphate. The volume in each flask was made

up to 10 c.c. with phosphate buffer at pH 7.2. Since all the histamine was taken from one ampoule, only one intracutaneous test (from the mixture in any one flask) was necessary to determine the activity of the histamine. One-tenth cubic centimeter of the mixture from any one flask was injected intracutaneously, and the size of the wheal was measured and recorded. The concentration of the histamine was 1:40,000, and the size of the wheal was usually from 16 to 18 mm. The flasks were then incubated at 37° C. under toluene for twenty-four hours. Starting with the mixture containing the largest amount of histaminase solution, 0.1 c.c. was injected intracutaneously from each flask until one of the sites produced a wheal of over 10 mm. The potency was fixed at the point between the injection which failed to produce a wheal and the one which just produced a wheal. By definition, a unit of histaminase is the quantity of enzyme that inactivates 1 mg. of histamine during twenty-four hours' incubation at 37° C. in a phosphate buffer solution of pH 7. Hence the expression of unitage may vary, depending on whether histamine base, histamine hydrochloride, or histamine phosphate is used as a standard. One milligram of histamine base actually contains 1 mg. of histamine, whereas 1 mg. of histamine hydrochloride involves 0.6 mg. of histamine and 1 mg. of histamine phosphate produces only 0.3 mg. of histamine. Therefore, the actual histamine ratio, according to molecular weights, is 3:2:1, respectively. In this work, since histamine phosphate was used, it is necessary to divide the units expressed by 2 in order to obtain unitage against histamine hydrochloride and by 3 in order to obtain unitage as represented by histamine base. In the future it might be expedient to express all units in relation to histamine base.

According to McHenry and Gavin,[4] extraction is best done by extracting one part of kidney powder with 18 parts of phosphate buffer. The solution is filtered and the powder is again extracted with a similar amount of phosphate buffer. In their procedure the filtrates were combined. This procedure was followed, but each of the filtrates was tested separately. The results obtained are indicated in Table I.

TABLE I

(Nitrogen is expressed as milligrams per cubic centimeter)

EXTRACTION	COLOR	POTENCY	PROTEIN NITROGEN	N.P.N.	TOTAL N
First	Amber	Over 10 units per cubic centimeter	0.90	0.29	1.19
Second	Light yellow	None	0.24	0.86	1.10

The following conclusions were drawn:

1. The potency is in a general way directly proportional to the depth of color of the original solution.

2. The second extraction had very little or no potency. It was, therefore, discarded as a step in the procedure.

3. The potency is related to the protein nitrogen fraction. The original extraction was finally modified so that each gram of powder was extracted with only 10 c.c. of phosphate buffer. By this method original solutions (filtrates), having from 5 to 75 units per cubic centimeter (average 12 units per cubic centimeter), were obtained.

PROPERTIES OF ORIGINAL SOLUTION (FIRST EXTRACTION)

I. The original solution can be clarified and sterilized by Seitz filtration without immediate loss in potency. It is clear, light to dark amber in color, has a strong odor of fresh animal tissue infusion, and is fairly stable at icebox temperature for about one to two months. However, solutions are rather unstable even at these temperatures, developing turbidities, flocculations, and precipitates, and generally losing a maximum of 50 per cent of their potency in from two to three months. One solution kept in the icebox for sixteen months developed a turbidity but maintained about 90 per cent of its original potency. Nevertheless, the solutions are sufficiently stable when fresh (about one month) for various in vitro experiments and for clinical use by intramuscular injection.

II. The solution is rather irritating and produces a distinct smart and pain on intramuscular injection, lasting ten minutes. An expedient maximum dose of 10 c.c. can be injected intramuscularly (average 120 units). Occasionally, one encounters the phenomenon described by Greenbaum[10] of an intense local reaction coming on in a few hours, but this is no contraindication to continued treatment. In one case a severe asthmatic attack was induced in ten minutes, but further injections failed to produce attacks in the same case.

III. The solution can be concentrated to a dry tan powder, without loss of potency, by fanning in a cellophane bag. This dried powder is more permanently stable than the solution and is readily soluble in water. It is quite possible to prepare and store this powder aseptically in ampoules by means of the Adtevac,[11] Lyophile,[12] Cryochem[13] process, or by some similar vacuum-refrigeration-concentration process. Due to lack of suitable equipment these latter procedures were not carried out.

IV. The effects of heat, hydrogen-ion concentration, and tryptic digestion confirm the report of McHenry and Gavin.[4] If the histamine-histaminase mixture was adjusted to a pH of 5.0 or less previous to incubation, the histaminase was irreversibly inactivated. In the tryptic digestion experiments the trypsin used had an index or activity of 3.3 by Gross' method.[14] A little of this trypsin was added to a solution of histaminase having a potency of 2 units per cubic centimeter and stored at icebox temperatures. An untreated control histaminase solution was similarly stored. After a few days the trypsin-treated histaminase and the control were incubated with histamine, and the titration was carried out on the virgin guinea pig uterus in the Dale bath. The usual skin titration was not carried out because injected trypsin has properties similar to those of snake venom.[15] The following results were found:

1. Trypsin solution itself did not stimulate the guinea pig uterus.
2. Trypsin-treated histaminase did not stimulate the uterine strip.
3. The control mixture (after incubation) did not cause contraction.
4. The histamine trypsin-treated histaminase mixture (after incubation) caused a marked contraction of the uterine strip.

This showed that the histamine in result 4 was not destroyed and that the trypsin had inactivated the histaminase even at icebox temperatures. Any temperature of over 60° C. inactivated histaminase irreversibly.

V. Attempts to purify the solution by ultrafiltration were not successful. A potent amber histaminase solution, containing 7 units per cubic centimeter, was ultrafiltered with nitrogen pressure through two layers of No. 600 cellophane. While the pH of the solution was 7.2, the clear Nile green ultrafiltrate had a pH over 8.0.

The green ultrafiltrate did not possess any potency and was protein free. The material left on the cellophane was dark brown in color, did not redissolve easily, contained a lot of protein, and had a potency of only 3.5 units per cubic centimeter. Dialysis experiments showed failure of the active material to pass through the cellophane bag into the external buffer.

VI. Attempts to purify the enzyme by ammonium sulfate precipitation, followed by iced acetone extraction, in most instances produced a total loss of potency.

VII. The concentration of histaminase in beef and lamb kidneys is much less than in hog kidneys. Repeated attempts showed a maximum value of 1 unit per 20 c.c. in beef kidney solution, and 1 unit per 3 c.c. in lamb kidney solution. Both of these solutions were light yellow in color.

VIII. In evaluating the potency of hog kidney flesh autolysates (without defatting), freshly ground hog kidneys were allowed to autolyze under toluene in the incubator at 37° C. for from seven to fourteen days. It was found that the histaminase potency was always less than that obtained from the kidney powder. For example, one batch of kidney flesh produced solutions having a potency of 2 units per cubic centimeter. The solution obtained from powder from the same batch had a potency of from 5 to 25 units per cubic centimeter.

IX. As a demonstration of the enzymatic property of histaminase, a solution having a potency of about 1 unit per cubic centimeter was incubated with 1 mg. of histamine phosphate for twenty-four hours and showed the usual slight cloudiness. Skin test showed that histamine had been destroyed. The mixture was clarified by filtration, and another 1 mg. of histamine phosphate was added. After twenty-four hours' incubation, the histamine was found to be inactivated. A further milligram of histamine was added, and in twenty-four hours it was found to be destroyed. This type of reaction is rather characteristic of enzymes, as contrasted with purely chemical reactions.[16]

X. The effects of histaminase and histamine on the human skin and organism, as revealed in this study, may be of some interest. Eight batches of hog kidney powder and one batch each of lamb and beef kidney powder were prepared. Each batch usually contained about 200 Gm. of dried powder. From each batch about five lots of solution were prepared. This made a minimum of 50 lots of solution in a period of eight months. Each lot had an average number of 10 intracutaneous tests done. Hence, over 500 tests with histamine, histaminase, and mixtures of these were done on my left forearm. At no time was there any indication of any local refractoriness to histamine. Nor was there any evidence of increased sensitivity. The same site could be reinjected at any time from fifteen minutes to months after the first injections and still respond with a marked wheal. For example, a fresh site was injected with 0.1 c.c. of 1:10,000 histamine and a wheal of 18 mm. was developed. In about thirty minutes, when the flare was subsiding and the edges of the wheal were

less pronounced, 0.1 c.c. of 1:1,000 histamine phosphate was injected into the same puncture. In a few minutes a larger wheal with pseudopods developed. During the injections for over eight months it was natural that a large amount of histaminase would be injected and absorbed. This had no effect whatsoever on the subsequent histamine skin reactions. Two further experiments were performed:

(1) A potent histaminase solution containing 5 units per cubic centimeter, was injected intracutaneously in three sites, 0.3 c.c. being used at each site. Tests with 1:10,000 histamine phosphate in fifteen minutes, four hours, and twenty-four hours (one site at a time) produced prompt and typical histamine wheals.

(2) A highly potent histaminase solution, containing 15 units per cubic centimeter, was injected in two sites, 1 c.c. being injected intracutaneously at each site. Eighteen hours later one site was tested with 1:40,000 histamine phosphate and produced a wheal of 18 mm.; the other site was tested with 1:1,000 histamine phosphate and produced a wheal of 25 mm.

On several occasions after extensive skin testing, there was some lassitude, back pain, and axillary tenderness. Part of this may have been due to the protein injected. On two occasions there was a neuritis involving the left shoulder and arm. The first was relieved by cessations of the injections and short wave diathermy. The second neuritis responded very promptly to ¾ grain of oral propadrine. Despite the numerous hog protein injections, no sensitivity to pork developed.

DISCUSSION

The field of enzyme chemistry has made extraordinary advances in the past ten years. Organic or "living" enzymes are extremely labile substances sensitive to heat, cold, temperature gradients, hydrogen-ion concentration, electrolytic and colloidal concentrations, and many other physical and chemical factors. In order to secure sufficient final yields of high potency and purity, one must begin with hundreds of grams of original material.[17] Enzyme chemists believe that they have recently isolated some of the enzymes in pure crystalline form after many years of intensive research.[17] The most refined present preparations of histaminase are as yet far from the pure state attained for these other enzymes. The histaminase solutions used for injections are conglomerations of numerous aqueous extractives of hog kidney and intestinal mucosa. Separation of proteins in these solutions is quite incomplete, and various enzymes, hormones, salts, and inorganic materials in the mixture have not yet been completely investigated. Therefore, though the mixture inactivates histamine, we do not know as yet the reason for its reported beneficial effects.[18] It must be realized that when the solution is given by injection, a large amount of nonspecific protein is given. In 10 c.c. there are about 9 mg. of protein nitrogen. Its beneficial effects by mouth (considering only the histaminase) are inexplicable in view of the in vitro experiments with tryptic digestion and the effect of pH below 5.0.

The enzymatic activity of solutions of histaminase seems definitely linked to the protein. Any attempt to remove all the protein results in inactivation

of the enzyme. This is further evidence that we are dealing with an enzyme in accordance with the present-day concept that organic enzymes are proteins or are closely allied to proteins.[16, 17]

Although different persons have varying sensitivities to intracutaneous histamine, most of them have approximately the same sensitivity as described in this study. Hence, a method of titration of histaminase potency in human beings, using suitably titrated and standardized recompensed donors, is offered for clinical trial. The method is apparently sufficiently sensitive to meet all but the most exacting requirements. By using histamine from the same sterile lot in all titrations, the number of control injections can be reduced.

It was found that even though a batch of kidney powder was thoroughly mixed, different lots of solution from the batch extracted under exactly the same conditions would have different potencies. Therefore, it is inadvisable to test only one lot at a time in evaluating a batch of powder. Each lot of solution must be titrated separately.

The kidney histaminase variation in members of a species, determined by McHenry and Gavin,[6] is compared with my findings in Table II.

TABLE II

(Figures represent milligrams of histamine base inactivated by 1 Gm. of kidney flesh)

ANIMAL	MC HENRY AND GAVIN	THIS WORK
Sheep	3.2	0.3 (lamb)
Ox	1.6	0.16 (steer)
Hog	3.6	10.0 average

It should be noted that only a small number of batches (one each) of lamb and beef kidney flesh and powder was prepared. Therefore, figures for lamb and steer may not be fully representative.

It has been found[4] that there is a latent period in vitro before any inactivation of histamine occurs. This enzymatic property of latency is further demonstrated in the experiments in which skin sites heavily inoculated with potent histaminase solution failed to prevent the whealing reaction of histamine. This lack of "protection" may have some clinical significance.

A word of caution concerning the incubation during titration is in order. During the course of the investigation a peculiar property of histamine was observed. If 1 mg. of histamine phosphate (1 c.c. of 1:1,000) was added to 9 c.c. of phosphate buffer at pH 7.2 and tested intracutaneously, a wheal of 18 to 20 mm. was obtained. If the flask was then incubated at 37° C. for twenty-four hours *without* added toluene, the fluid would become somewhat cloudy. A skin test now would frequently show no histamine effect (lack of whealing), and the guinea pig uterus in the Dale bath would not respond. However, if a thin layer of toluene was added to the histamine in phosphate buffer previous to incubation, the solution would preserve its clarity and also its whealing effect indefinitely. The culture from the cloudy solution showed a gram-positive bacillus. Undoubtedly, the described destructive effect on histamine was due to the action of these air bacilli upon it.

Another paper will deal with the clinical results attained by the injection of potent histaminase solutions.[19]

SUMMARY

Further laboratory studies in the preparation, titration, properties, and purification of the enzyme-system histaminase are reported. A more accurate method of titration on human skin is described.

The physical properties of phosphate buffer solutions of histaminase and the effects of heat, pH, and trypsin are outlined. Several methods of purification and concentration are evaluated.

Further evidence that histaminase is an enzyme is adduced.

Some of the local and general effects of histamine and histaminase used over a long period of time are related.

The need for much more laboratory investigation in the purification and evaluation of the various components of the crude mixtures now available is stressed.

I wish to express my thanks to Mr. Cully, of Figge and Hutwelker, New York City, who so kindly supplied an unlimited number of fresh hog kidneys.

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160 WEST MERRICK ROAD

HISTAMINASE

Results of Treatment by Intramuscular Injection

Maurice Vaisberg, M.D., Baldwin, N. Y.

IN THE past five years interest has been stimulated in the histaminase treatment of allergic and allied conditions. A considerable literature has thus come into existence. Excellent partial reviews of previous clinical work are to be found in several articles.[1-5] Other papers[6-10] have more complete surveys and extended bibliography. In this presentation, for the sake of brevity, very little reference will be made to other work.

The histaminase solutions used in this study were all prepared from fresh kidney tissue in the laboratory, as described in the paper on laboratory studies.[12] The solution used was the first phosphate buffer extraction of hog kidney powder, varied from light to dark amber in color, and was injected intramuscularly in all cases. Its potency ranged from 5 to 25 units per cubic centimeter (as against histamine phosphate). The usual maximum given at one time was generally 10 c.c. Early in each case injections were usually given twice a week and later, if the case improved, once a week.

The following types and numbers of cases were treated:

Asthma	7	(2 of these had neurodermatitis)
Neurodermatitis	4	(2 of these were of above asthma group)
Vasomotor rhinitis	3	
Chronic urticaria	2	
Serum sickness	1 each	
Dermatographia		
Pruritus		
Migraine		
Heat allergy		
Total	19	cases

It is probably best to analyze these cases by tabulations, giving the case identification, duration of symptoms, average dose per week, number of weeks that histaminase was given, length of time of observation from inception of histaminase treatment and outcome. Each tabulation has appended a discussion of special features involved.

Case 5. The first series of injections was given simultaneously with nasal antrum washings. The patient improved rapidly, both as concerned asthma and neurodermatitis. But a relapse occurred despite continued treatment necessitating cessation of injections. During the period of nontherapy she rapidly got much worse. It was difficult to determine whether the histaminase

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injections or the antrum washings were responsible for the initial improvement. The second series of injections starting thirteen weeks after the first series was given alone with temporary improvement only.

Case 6. Under treatment there were fewer attacks of dyspnea, but a moderate cough continued. Despite relief the ingestion of interdicted foods would produce slight wheezing.

TABLE I

ASTHMA

CASE	DURATION	DOSE PER WEEK IN UNITS	NO. OF WEEKS	TIME OF OBSERVATION IN MONTHS	OUTCOME
1. S. G. Simple asthma	16 yr.	40	10	2½	Helped at first, worse later
2. R. B. Simple asthma	5 yr.	30	2	10	Made much worse
3. J. B. Simple asthma	2 yr.	20	10	2½	Helped at first, worse later
4. L. K. With bronchiectasis	5 yr.	80	4	6	No better
5. E. K. Plus neurodermatitis	Life	70 0 90	10 13 2	5	Improved, then worse, etc. *See* Discussion
6. P. R. Plus infection	12 yr.	90	12	3	Better. *See* Discussion
7. A. B. Asthma Neurodermatitis	Life 6 mo.				*See* under Case 2, Neurodermatitis group

TABLE II

NEURODERMATITIS

CASE	DURATION	DOSE PER WEEK IN UNITS	NO. OF WEEKS	TIME OF OBSERVATION IN MONTHS	OUTCOME
1. E. K.	*See* Case 5 under Asthma				
2. A. B.	6 mo.	50 100	2 2	6	Improved at first, then worse. *See* Discussion
3. J. B.	1.5 yr.	556	1	2	Slight temporary aid. *See* Discussion
4. E. L.	Life	900	2	2	Temporary aid. *See* Discussion

Case 2. The asthma was controlled very well by routine allergic treatment, but the neurodermatitis persisted. The first injection of histaminase completely relieved the pruritus for a few days, but further injections caused recurrence of asthma and itching. A second series of injections twelve weeks after the first failed to produce any relief.

Case 3. Relief of pruritus was sporadic. The lesions cleared slightly. On release from the hospital there was a severe recurrence.

Case 4. The pruritus was variably relieved. The lesions cleared rapidly. The patient was treated in the hospital and relapsed badly four days after leaving hospital. There was, however, a marked psychic element in this case.

Case 2. The patient developed a tremendous wheal to a preliminary intracutaneous test precluding the use of hog histaminase. A preparation of lamb kidney histaminase having a potency of 1 unit per 3 c.c. was administered.

TABLE III

VASOMOTOR RHINITIS

CASE	DURATION	DOSE PER WEEK IN UNITS	NO. OF WEEKS	TIME OF OBSERVATION IN MONTHS	OUTCOME
1. H. S.	5 yr.	45	4	1¼	No help
2. G. D.	5 yr.	44	6	5	No help
3. J. D.	5 yr.	71	5	5	No help

TABLE IV

CHRONIC URTICARIA

CASE	DURATION	DOSE PER WEEK IN UNITS	NO. OF WEEKS	TIME OF OBSERVATION IN MONTHS	OUTCOME
1. N. G. Female	1 yr.	158	2	4	No help
2. N. G. Male	3 yr.	3 (lamb)	11	7	No help. *See* Discussion

Three units were given for eleven weeks without any help. Histamine injections produced a gratifying amelioration of all pruritus. However, the hives continued to appear, although they did not itch. This patient was sensitive to cold as demonstrated by the ice test.

TABLE V

MISCELLANEOUS

CASE	DURATION	TREATMENT	TIME OF OBSERVATION IN MONTHS	OUTCOME
E. S. Serum sickness	4 days	1 injection of 60 units	½	Permanent relief in 3 hours
E. J. Dermatographia	3 yr.	300 units in 2 weeks	2	No help
J. B. Pruritus	1 mo.	1 injection of 60 units	6	Permanent relief in few hours
G. T. Migraine	2 yr.	2 injections of 75 units each on successive days	2 days	No help
A. V. Heat allergy	5 yr.	*See* Discussion	12	Helped second time with large doses

The case of heat allergy (A. V.) was previously described in some preliminary work on histaminase.[11] At that time very small doses of histaminase (less than 1 unit) relieved the swelling of the feet occasioned by hot weather. The following summer nothing was done. The heat of the 1940 summer produced painful ankle edema again. It was now necessary to give about 160 units in two weeks to produce relief. The first injection induced an immediate violent asthmatic attack and a severe local reaction, but subsequent injections elicited only the local reaction in diminishing severity.

COMMENT

In all patients a preliminary intracutaneous test for pork sensitivity was performed with the solution. In one patient (2. N.G., chronic urticaria) the

local reaction was so large that lamb histaminase was substituted. In general, a mild or even moderate local reaction is no contraindication to treatment, although the usual care must be exercised. One case (A. V., heat allergy) developed an immediate constitutional reaction in ten minutes. One patient (A. B., asthma-neurodermatitis) had a delayed general effect of asthma in four hours after injection. Neither of these patients developed asthma on subsequent injections. Other general reactions, as reported by others, such as vertigo, nausea, flatulence, laxation, or headache;[7] and fever, malaise, or joint pains[6] were not observed in this series.

Local reactions, varying from mild redness and swelling to those described by Greenbaum,[14] were seen in several cases. The latter consisted of erythematopapular areas at the site of injection, several inches across, with raised margins, marked itching, and local heat. After several inoculations the severity would diminish. There was never any evidence of necrosis. Local pain lasting ten minutes was elicited in all cases immediately upon injection of histaminase solution.

In evaluating any beneficial results one must take into account the well-known tendency of allergic diseases to improve and retrogress spontaneously. Hence, in a small series of cases as presented here, final conclusions cannot be drawn, and only the most meager clues or indications can be found as to the efficacy of this type of medication. For final statistical evaluation in this type of therapy, considering both the unknown quantities in the disease and in the medication, a very large series of cases with adequate controls (both auto- and hetero-) is indicated. In addition, any possible effect of nonspecific protein cannot be minimized. It should be remembered that each cubic centimeter of solution contains approximately 0.9 mg. of protein nitrogen.

Of the six cases treated for asthma, one was considerably improved, three were temporarily relieved, and two were not improved. One of the latter had a well-established bronchiectasis. Commenting, one could say that nonspecific protein might have given similar results.

Of the four cases of neurodermatitis, two (A. B. and E. K.) had variable relief, i.e., they were helped at one time and not at another; and two (J. B. and E. L.) who were hospitalized might have improved just from the hospital stay. Results attained here might have been obtained by other measures, such as calcium intravenously, bed rest, and even suggestion (hypnosis). However, the outstanding feature in all cases of pruritus, of whatever origin, where the medication did help, was the dramatic relief of itching in several hours.

None of the three cases of vasomotor rhinitis had the slightest relief.

Two cases of chronic urticaria had no relief whatsoever. This is in agreement with the much larger series of Piness and Miller,[13] who employed an oral preparation.

One case of serum sickness responded dramatically. This is in accord with the work of Foshay and Hagebusch,[1] although they gave the preparation orally and their injections in much smaller doses.

There was no response in either the one case of dermatographia or in the single case of migraine.

The ankle edema due to heat required a much greater dose the second summer of treatment than the first.

It is somewhat difficult to correlate these results of intramuscular injections with those of other observers who have given the oral preparation, in view of the experiments in the laboratory presentation[12] which demonstrated that histaminase is inactivated by trypsin. Possibly this may explain the advocated necessity of larger doses orally.

As tentative conclusions in this study it might be stated that histaminase by injection, using our present preparations, may prove to be of value in resistant serum sickness, in idiopathic pruritus, and in heat and cold allergy. It may have a limited usefulness in neurodermatitis. It has an unpredictable effect on simple asthma, and no effect on vasomotor rhinitis, dermatographia, migraine, and chronic urticaria. It was not tried in hay fever.

Various lines of evidence, considered logically, lead to the impression that the beneficial effects of histaminase observed by various authors who have used the preparation both orally and by injection, are due to something else in the preparation other than the enzyme-system histaminase. The successful results reported by Altose,[7] Goldberg,[6] Foshay and Hagebusch,[1] Roth and Rynearson,[15] Prickman and co-workers,[16] and Roth and Horton,[17] in its oral administration, despite the fact that histaminase is destroyed by stomach acidity, pepsin, and trypsin, indicate that there is something *other than* the enzyme which is responsible for the improvement. The work of Fox and Harned[18] in which they used a cresolated solution of hog nasal mucosal extract by injection for chronic and acute rhinosinusitis with good results, point in the same direction. Cresol is known to destroy histaminase.[19] The very properties of the enzyme-system histaminase militate against its ability to relieve the ill effects of the theoretical instantaneous release of histamine in anaphylactic shock and allergic reactions. The destruction of histamine by histaminase is not instantaneous and is generally quite slow. It is extremely doubtful that a sudden release of histamine in the body could be counteracted by administration of the enzyme before toxic effects had been produced. Thus, logically one is inclined to believe that any beneficial action reported in any condition for which histaminase was given orally or by injections was due to something else in the protein of the hog kidney or intestinal mucosa.

Finally, much more clinical work must be done with a potent, highly purified histaminase before the validity of any claims as to its efficacy as histaminase alone can be firmly established. Naturally, this involves further laboratory work in the purification and isolation of the enzyme and then the consistent evaluation of its effects in animals. Only when we have done these can we correctly interpret the separate effects of the histaminase and the other protein and nonprotein constituents of the solution.

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REGIONAL AND SEASONAL VARIATIONS IN THE SERUM CHOLINE-ESTERASE OF HUMAN BEINGS AND DOGS*

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Hall and Lucas[1] in 1937 reported determinations of serum choline-esterase in 40 normal persons. They found values ranging from 1.32 to 3.61, with an average of 2.50. While determining serum esterase activity in a series of patients with peptic ulcer,[2] we had occasion to check normal values in this region. Determinations were made on 50 normal persons (students and secretaries). In this series were found values varying from 2.94 to 6.58, with an average of 4.72. The great difference between these values and those of Hall and Lucas assumes much importance in the comparison of pathologic cases with normals.

We are unable to offer any satisfactory explanation for the above discrepancy, except that possibility that a climatic or geographic factor is responsible. Without implying that choline-esterase is related to thyroid activity, the fact may be cited that Ring[3] has shown that exposure to temperatures of 0° to 5° C. for periods of three weeks or more brings about a definite increase in thyroid activity in rats. Further, it is generally recognized that the normal standards established for basal metabolic rates in the colder climates are

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approximately 15 per cent above those encountered in the southern states. However, if climatic conditions are responsible for the observed differences in choline esterase values, it appears that they produce these changes very slowly in that no significant seasonal variations have been observed in our series nor have we been able to demonstrate seasonal variations in serum esterase activity in dogs.

To find whether sudden wide changes in body temperatures alter serum choline-esterase values, we have made determinations of choline-esterase activity in a series of six persons who were being subjected to artificial fever therapy. Blood samples were obtained from the patient immediately before entering the machine and again after five to seven hours during which time a body temperature of 104.6° to 106.6° F. had been attained.* The procedure revealed no consistent change in esterase values. The average value obtained in 9 determinations before the induction of fever was 4.21, whereas the average value afterward was 4.37.

TABLE I

MEAN WEEKLY CHOLINE-ESTERASE ACTIVITY IN FIVE DOGS FOR A PERIOD OF ONE YEAR

DATE	MEAN VALUE	STANDARD DEVIATION	DATE	MEAN VALUE	STANDARD DEVIATION
1/18/38	1.75	0.19	7/29/38	1.90	0.22
1/25/38	1.95	0.21	8/ 5/38	1.72	0.18
2/ 1/38	1.98	0.42	8/16/38	1.98	0.10
2/ 7/38	1.81	0.26	8/20/38	1.88	0.25
2/14/38	1.77	0.32	8/26/38	1.82	0.16
2/21/38	1.57	0.14	9/ 2/38	2.03	0.32
2/28/38	1.78	0.08	9/ 9/38	2.00	0.26
3/ 7/38	2.14	0.14	9/16/38	2.08	0.10
3/14/38	2.16	0.30	9/23/38	1.77	0.23
3/21/38	2.53	0.15	9/30/38	1.73	0.28
3/28/38	3.12	0.57	10/ 7/38	1.68	0.13
4/ 8/38	1.93	0.26	10/14/38	1.71	0.24
4/15/38	1.77	0.33	10/21/38	1.75	0.09
4/22/38	2.14	0.21	10/28/38	1.66	0.26
4/29/38	2.11	0.16	11/ 4/38	1.86	0.16
5/ 6/38	1.77	0.18	11/11/38	1.62	0.13
5/13/38	2.08	0.23	11/18/38	1.65	0.05
5/20/38	2.36	0.20	11/25/38	1.82	0.24
5/27/38	2.70	0.42	12/ 2/38	1.79	0.17
6/ 3/38	2.41	0.15	12/ 9/38	1.88	0.16
6/10/38	2.14	0.28	12/16/38	1.82	0.34
6/17/38	2.05	0.12	12/23/38	1.03	0.25
6/24/38	1.82	0.25	12/30/38	1.95	0.19
7/ 1/38	2.05	0.33	1/ 2/39	2.05	0.08
7/ 8/38	1.85	0.21	1/ 9/39	2.00	0.36
7/15/38	1.65	0.21	1/16/39	1.97	0.13
7/22/38	1.56	0.15			

In order to check this wide variation of serum choline-esterase values in human beings, we have made determinations of serum choline-esterase activity at weekly intervals for one year in a series of five dogs. We felt such a comparison would cross check these differences and also bring out any seasonal variations. Analyses were carried out according to the technique described by Hall and Lucas.[1] The values obtained are summarized in Table I in which, to avoid excessive length, is recorded only the mean value for the group of five animals for each date, together with the calculated standard deviation for that date.

*In one patient two treatments caused an elevation of body temperature to only 101° and 101.4° F.

Daily temperature records reveal that the plateau of the temperature curve extends from April 29 to September 16. The mean esterase value for this interval (twenty-one weeks) is 1.99, with a standard deviation* of 0.23. The mean value for the remainder of the twelve-month interval is 1.88, with a standard deviation of 0.33. Thus the values are slightly higher during the warmer period, but the difference is insignificant. It is worthy of note that, whereas the mean value for serum esterase activity (1.92) for this series of dogs is less than one-half that found in our series of normal human beings (4.72) in whom determinations were made concurrently, Hall and Lucas,[1] and Hall and Ettinger[4] find no great difference in choline-esterase activity in the two species.

SUMMARY AND CONCLUSIONS

1. Normal human serum choline-esterase values in Texas appear to be approximately twice those reported from Toronto, but no consistent seasonal variations have been noted.

2. Elevation of body temperature by artificial fever therapy does not alter human serum choline-esterase values.

3. There is no significant seasonal variation in serum choline-esterase activity in normal dogs.

4. Whereas there appears to be a geographic or climatic difference in serum choline-esterase activity in the human being, values reported here for dogs approximate those reported from Toronto.

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*Standard deviation is calculated by the formula $\sigma = \frac{D^2}{(n-1)}$ where D^2 equals the sum of the squares of the difference of each value from the mean value of the group. n equals number of determinations in the group.

THE FRACTIONATION OF THE BLOOD IODINE*

I. Findings in Patients With Normal Thyroid Function and With Hypothyroidism

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EXTENSIVE investigation has now firmly established the close relation between thyroid function and the quantity of iodine ordinarily circulating within the blood. In the absence of iodine administration in any form the blood iodine is usually decreased in patients with underactive thyroid glands and is increased in those with overactive thyroid glands. The normal range for the iodine content of whole blood varies considerably with the method used for its determination; nevertheless, all methods usually show an increased or decreased blood iodine in hyperthyroidism or hypothyroidism, respectively. These widely reported studies, and particularly the conclusions drawn from them, have formed the groundwork for further insight into the nature of disturbed thyroid function. Moreover, they have disclosed that significant knowledge may be derived from a careful differentiation of the various iodine compounds found in the blood and an interpretation of their possible metabolic significance.

At present, however, the status of both qualitative and quantitative microchemistry is such that the accurate identification and quantitation of these individual iodine-containing substances is hardly practicable. Nevertheless, there is a constant and ever-increasing attempt at the separation and identification of these compounds with an especial effort directed toward the *quantitative determination of the circulating thyroid hormone.*

Various methods have been employed in several attempts to quantitate the circulating thyroid hormone. Barkan[1] attempted to separate the "*organic*" blood iodine from the "*inorganic*" by precipitation with silver nitrate. Techniques based on the precipitation of thyreoglobulin by a specific antithyreoglobulin serum have been applied by other investigators.[2-7] Alcohol has been employed by Perkin and Hurxthal[8] and Trevorrow[11] as a precipitant in order to separate the blood iodine into soluble and insoluble fractions. Other workers[9-11] have employed acetone as the precipitating and thus the fractionating agent. McClendon and his associates[12-15, 17] employ methanol followed by acetone in their procedure for the fractionation of blood and tissue iodine.

In this report an attempt is made to evaluate the fractionation of the blood iodine in euthyroid persons of this region and in hypothyroid patients. We have employed an acetone procedure. Special attention is paid the insoluble fraction. We *presume* that this fraction contains the circulating thyroid hormone.

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TABLE I

EUTHYROID INDIVIDUALS

(Fractionated Blood Iodine)

NO.	AGE	SEX	B.M.R. PER CENT	ACETONE AND WATER SOLUBLE I_2	INSOLUBLE I_2	TOTAL I_2	DIAGNOSIS
1	29	M	Plus 20	3.64	0.51	4.15	8 days preoperative for right indirect inguinal hernia
			Minus 3	3.20	0.51	3.71	7 days postoperative to right indirect inguinal hernia
2	7	M	Minus 9	3.70	0.53	4.23	Normal
3	25	M	Minus 8	5.14	1.34	6.48	Normal 7/24/39
	26		Minus 7	3.13	0.59	3.72	Normal 7/30/40
	26		Minus 12	2.96	0.54	3.50	Normal 8/6/40
4	34	M	Plus 16	3.20	1.20	4.40	Acromegaly 6/18/40
			Plus 12	4.14	0.56	4.70	Acromegaly 8/22/40
5	33	F	Minus 2	3.81	0.63	4.44	Normal
6	23	F	Plus 1	3.30	0.65	3.95	"Nervousness"
7	42	M	Minus 16	2.80	0.68	3.48	Chronic irritable colon; "nervous exhaustion"
8	55	F	–	3.47	0.76	4.23	Skull fracture
9	46	M	Plus 9	3.22	0.93	4.15	Carcinomatosis
10	22	M	Minus 5	3.56	0.94	4.50	Hypoparathyroidism
11	32	M	Minus 10	2.62	1.01	3.63	Normal
12	49	M	Plus 16	2.96	1.02	3.98	Chronic ulcerative colitis
13	44	F	Minus 10	3.56	1.18	4.74	7 days postoperative to herniorrhaphy 12/29/39
			Minus 3	6.00	1.10	7.10	9/19/40
14	24	M	Minus 14	3.74	1.26	5.00	Normal
15	25	M	Plus 6	3.16	1.27	4.43	Arrested tuberculosis
16	52	M	Plus 11	3.80	1.27	5.07	"Nervousness"; "anxiety"
Av.	33		Plus 1	3.58	0.88	4.45	

All iodine values are in micrograms per 100 c.c.

TABLE II

HYPOTHYROIDISM

(Fractionated Blood Iodine)

NO.	AGE	SEX	B.M.R. PER CENT	ACETONE AND WATER SOLUBLE I_2	INSOLUBLE I_2	TOTAL I_2	DIAGNOSIS
1	52	M	Minus 28	1.94	0.23	2.17	Hypothyroidism
2	33	M	Minus 15	2.36	0.32	2.68	Hypothyroidism
3	38	F	0	2.62	0.42	3.04	Hypothyroidism; "nervousness"
4	34	F	Minus 19	3.62	0.46	4.08	Hypothyroidism
5	11	M	Minus 24	3.64	0.46	4.10	Hypothyroidism
6	24	M	Minus 13	3.89	0.51	4.40	Hypothyroidism
7	33	M	Minus 10	2.80	0.51	3.31	Mild hypothyroidism
8	45	M	Minus 20	4.66	0.58	5.24	Hypothyroidism
9	26	M	Minus 1	2.46	0.59	3.05	Mild hypothyroidism, obesity 8/16/40
			Minus 4	2.79	0.59	3.38	Mild hypothyroidism, obesity 11/4/40
10	39	F	Minus 24	3.38	0.59	3.97	Mild hypothyroidism
11	42	M	Minus 14	3.50	0.63	4.12	Mild hypothyroidism
Av.	35		Minus 14	3.14	0.49	3.62	

All iodine values are in micrograms per cent.

METHOD

Fifty milliliters of oxalated blood is pipetted into three volumes of iodine-free absolute acetone. During the addition the mixture is agitated by rotating the flask in order to insure an even and fine precipitation of the blood proteins. The blood-acetone mixture is then allowed to stand at laboratory temperature for twelve hours to insure complete precipitation of the blood proteins and the maximum diffusion of acetone-soluble substances of the blood into the solvent.

The precipitate is then separated from the solution by filtration through a Buchner funnel, using a 11 cm. (589) "Blue Ribbon" Schleicher and Schull filter paper. The precipitate is then washed with 500 ml. of a 75 per cent acetone-water solution. It is further washed with 500 ml. of double-distilled iodine-free water. The filtrate and washings are pooled and analyzed for total iodine content. This is termed the acetone and water *soluble fraction*.

The remaining precipitate containing the acetone and water *insoluble fraction* is removed quantitatively from the funnel and analyzed for total iodine. The Matthews, Curtis, and Brode[16] modification of the Leipert procedure was employed in all our determinations.

OBSERVATIONS

In 20 determinations made on 16 patients without detectable evidence of thyroid disease, the basal metabolic rate averaged plus 1, ranging from minus 16 to plus 20 (Table I). The whole blood iodine of this group varied from 3.5 to 7.1 micrograms per 100 c.c. averaging 4.45, which is normal for persons in this region on a nonconstant food regimen. The acetone and water *insoluble fraction* averaged 0.88, with variations of from 0.51 to 1.34 micrograms per 100 c.c. The acetone and water *insoluble fraction* does not closely parallel the basal metabolic rate. However, upon analysis of the individual patients with particular reference to the cause of the increased oxygen consumption, a clearer view of the significance of the *insoluble fraction* and its value as a differential diagnostic agent becomes apparent. In Tables I and III it may be noted that persons with increased basal metabolic rates not clinically referable to the thyroid gland present a normal or even lower than normal *insoluble fraction*.

The *insoluble fraction* even fluctuates in the same person (Table I). Moreover, this fluctuation shows only little relationship to the concurrent fluctuations in the basal metabolic rate. Although these individual fluctuations remain within the limits of what we have found to be the normal range for the insoluble blood iodine, they are difficult to comprehend without assuming an unexpectedly wide normal range in the circulating thyroid hormone. All determinations were made on bloods drawn in the morning in the postabsorptive state.

Table II presents a series of determinations made on patients with definite clinical evidence of hypothyroidism. The basal metabolic rate in this group averages minus 14, with variations from 0 to minus 28 per cent. The whole blood-iodine range is from 2.2 to 5.2, with an average of 3.6 micrograms per 100 c.c. The *insoluble fraction* averages 0.49, with variations from 0.23 in a patient with unusually well-developed hypothyroidism to 0.63 microgram per

100 c.c. in a patient with moderate but clinically obvious hypothyroidism. The upper limit of the insoluble blood iodine in this series is thus seen to overlap the lower limit of the insoluble blood iodine of the series of patients with normal thyroid function presented in Table I. (Fig. 1). However, this overlapping of

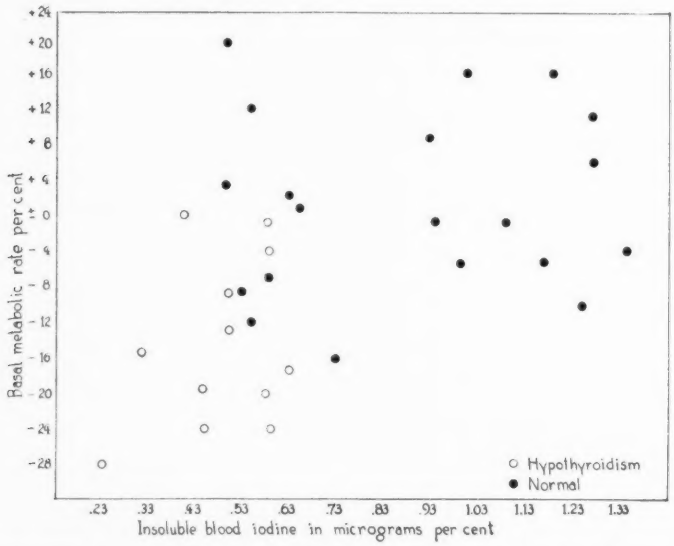

Fig. 1.—A comparison of the ranges of the insoluble blood iodine fraction in patients with normal thyroid function and with hypothyroidism.

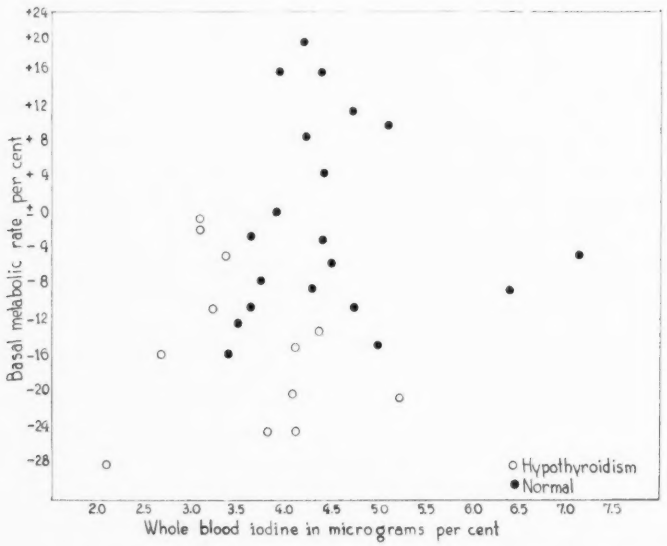

Fig. 2.—A comparison of the ranges of the whole blood iodine in patients with normal thyroid function and with hypothyroidism.

the insoluble blood iodine is not as extensive as that of the total blood iodine of the same two series (Fig. 2). Due to theoretical variation in the sensitivity of individuals to the thyroid hormone, as well as from their variable physiologic requirements for the hormone, a certain amount of overlapping might even be

expected, especially in the borderline cases. *The insoluble blood iodine is consistently low in patients with hypothyroidism.*

Four euthyroid individuals with cardiovascular disease (Table III) showed a range in basal metabolic rate of from plus 9 to plus 46 per cent. Two had a normal blood iodine, and two had a total blood iodine below normal. In all except the one with uncomplicated essential hypertension, the insoluble blood iodine was below the lowest limit of normal. The patient with essential hypertension presented a normal insoluble blood iodine.

TABLE III

PATIENTS WITH DEFINITE CARDIOVASCULAR DISEASE AND WITHOUT CLINICAL EVIDENCE OF THYROID DISEASE

(Fractionated Blood Iodine)

NO.	AGE	SEX	B.M.R. PER CENT	ACETONE AND WATER SOLUBLE I_2	INSOLUABLE I_2	TOTAL I_2	DIAGNOSIS
1	65	F	Plus 29	2.88	0.42	3.30	Hypertension; congestive heart failure; (16 months after subtotal thyroidectomy for diffuse hyperplastic goiter with nodules)
2	72	M	Plus 9	2.70	0.42	3.12	General arteriosclerosis; arteriosclerotic heart disease
3	36	M	Plus 46	4.40	0.44	4.84	Hypertensive cardiovascular disease; early congestive failure
4	21	F	Plus 24	3.63	0.71	4.34	Essential hypertension without complications
Av.	48		Plus 27	3.40	0.50	3.90	

All iodine values are in micrograms per 100 c.c.

We interpret these findings (Table III), not as hypothyroidism masked by hypertension, but as a subdued thyroid secretion compensatory to the increased supply of oxygen to the tissues as a result of the hypertensive circulation. If the thyroid hormone acts as a catalyst, increasing the utilization of oxygen by the individual tissues, and the tissues are already receiving, through an increased circulation, an abundance of oxygen for their metabolic activity, then the thyroid gland may theoretically, at least, compensate by decreasing the supply of circulating thyroid hormone. Although this series is too small from which to conclude, the consistently low insoluble fraction is apparently of value in differentiating hypertension from hyperthyroidism.

SUMMARY AND CONCLUSIONS

A method for the fractionation of the blood iodine into an acetone and water *soluble* fraction and an acetone and water *insoluble* fraction is presented.

The acetone and water *insoluble blood iodine* averages 0.88, with variations of from 0.51 to 1.34 micrograms per 100 c.c. in euthyroid persons without cardiovascular disease.

The acetone and water *insoluble blood iodine* is consistently low in hypothyroidism and ranges from 0.23 to 0.63, with an average of 0.49 microgram per 100 c.c. The *insoluble blood iodine* in hypothyroidism does not show as extensive overlapping into the normal range as does the whole blood iodine.

Hypertensive individuals that are clinically euthyroid present a low normal to lower than normal *insoluble blood iodine*. The low *insoluble* fraction of hypertensive patients may prove to be of diagnostic value in differentiating uncomplicated hypertension from hyperthyroidism. The *insoluble blood iodine* fluctuates individually.

The acetone and water *insoluble blood iodine*, as determined by the method presented, would appear to contain the circulating thyroid hormone.

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VALUES FOR ACETYLCHOLINE ESTERASE IN THE BLOOD SERUM OF NORMAL PERSONS AND PATIENTS WITH VARIOUS DISEASES*

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THE presence of an enzyme of the blood capable of hydrolyzing acetylcholine was first suggested by Dale,[1] but not until eighteen years later was its specificity definitely demonstrated by the Stedmans and Easson.[2] Under the leadership of Stedman and his associates rapid progress was made in the study of the physiologic and chemical properties of acetylcholine esterase, but the possible clinical importance of this new enzyme received little attention. Several of the earlier clinical investigators observed wide variation of the esterase activity among various persons on whom studies were carried out over periods of many weeks[3, 4] and for the most part were unable to correlate the acetylcholine esterase activity of the serum with any physiologic or pathologic condition.[5]

Within the past few years, however, several reports have appeared which suggest that the acetylcholine esterase activity of the blood serum may be altered in several clinical syndromes. Antopol and his associates,[6, 7] and McArdle,[8] reported low values for acetylcholine esterase in diseases of the liver and biliary tract, anemia, and hyperpyrexia. Milhorat[5] considered the low values in his group of 109 patients to be the result of debility or severe cachexia, as did Jones and Stadie.[9]

The present study was undertaken to determine if possible the clinical significance of low or high values of acetylcholine esterase in the blood serum of man. The values for acetylcholine esterase were obtained at a constant temperature of 30° C. by utilizing the Cameron glass electrode assembly. Twenty cubic centimeters of the substrate of acetylcholine bromide, containing 2 mg. per cubic centimeter, were placed in a vessel which was surrounded by a continuously flowing current of water at 30° C. The substrate was brought to a pH value of 8.5 by the addition of approximately one drop of tenth-normal solution of sodium hydroxide. To this was added 0.2 c.c. of serum, and with manual stirring, enough hundredth normal solution of sodium hydroxide was added at intervals of five minutes for a period of thirty minutes to maintain the pH value of the substrate at 8.5. A microburette was used for this purpose. A control or blank determination was run from which serum was omitted; the value obtained was subtracted from the value obtained with the serum. Values are expressed as the total cubic centimeters of hundredth normal solution of sodium hydroxide necessary to maintain the substrate at pH 8.5 over a period of thirty minutes when 0.2 c.c. of serum is used.

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VALUES IN BLOOD SERUM OF NORMAL PERSONS

Values for acetylcholine esterase in the blood serum of 47 normal men and 38 normal women were determined, a single determination for each individual being utilized for this purpose. Among the men the values ranged from 0.91 to 3.13, with an average of 2.06 ± 0.07 and a standard deviation of 0.48, and among the women from 0.90 to 2.99, with an average of 1.72 ± 0.08 and a standard deviation of 0.50. While the variability was essentially the same in the two sexes, the values in the serum of the women were definitely lower than among the men. The average value for the 85 men and women together was 1.90 and the standard deviation was 0.53. If we take a commonly utilized rule of statistics to accept plus or minus twice the standard deviation as the limits of significance (which will include 95 per cent of the cases), we can take 1.90 ± 1.06 or from about 0.85 to 3.0 as acceptable limits of normal variability.

TABLE I

VALUES FOR ACETYLCHOLINE ESTERASE IN A MISCELLANEOUS GROUP OF 24 PATIENTS

DIAGNOSIS	ACETYLCHOLINE ESTERASE (C.C.)*
Chronic nervous exhaustion	1.63
Erythromelalgia	2.45
Chronic duodenal ulcer with obstruction	0.76
Acute gouty arthritis and sinus bradycardia	1.95-1.65
Chronic duodenal ulcer	1.38
Epilepsy with periodic paralysis	
Between attacks	1.60
During an attack	1.65
Diabetes mellitus, grade 3	1.86
Obesity	2.31
Urticaria and angioneurotic edema	2.04-2.20
Postural hypotension	1.00
Sprue	1.64
Sprue	1.77-1.32
Average in first 12 cases	1.70
Chronic nervous exhaustion	1.05
Chronic nervous exhaustion	1.49
Anxiety neurosis	1.02
Psychoneurosis	1.67
Lipoma of neck	1.51
Indeterminate	1.13
Backache	1.72
Carcinoma of breast with pulmonary metastasis	1.70
Atopic eczema	1.26
Duodenal ulcer	1.73
Syphilis of the central nervous system; tabes dorsalis; causalgia	1.55
Sprue	0.92
Average in second 12 cases	1.40

*Cubic centimeters of 0.01 normal solution of sodium hydroxide.

Fluctuations in Values for Acetylcholine Esterase in Same Person.—The results given thus far are for one determination made for each of a number of individuals. The variation, therefore, represents the interindividual variability. In addition to this it was thought desirable to obtain some information concerning the variation of different determinations made for the same individual, that is, the intra-individual variation. Daily estimations were made on the serum of two normal persons. For one, six successive daily determinations had a mean of 0.86 ± 0.02 and a standard deviation of 0.06. For the other, seven

determinations had a mean of 1.17 ± 0.05 and a standard deviation of 0.14. The standard deviation measuring the intra-individual variability judged by these two persons together was 0.11. Thus it is seen that the intra-individual variability is about a fifth of the interindividual variability.

VALUES ASSOCIATED WITH VARIOUS DISEASES

Miscellaneous Group of Cases.—Values for acetylcholine esterase were determined in 24 patients in whom a variety of conditions was present. These included chronic nervous exhaustion, neurosis, epilepsy, obesity, urticaria, angioneurotic edema, duodenal ulcer, diabetes mellitus, syphilis of the central nervous system, sprue, postural hypotension, and arthritis (Table I). The values with one exception were within the range of normality (0.85 to 3.00), and the exception is not significant.

Hypertension.—Determinations of acetylcholine esterase were made in 13 patients with hypertension. The hypertension was of all grades of severity. The values ranged from 1.17 to 2.51 and averaged 1.58. Hypertension apparently does not produce changes in values for acetylcholine esterase.

Cardiac Disorders.—Values for acetylcholine esterase were determined in 12 patients with cardiac disorder. Our interest in this group was prompted by the possibility that the acetylcholine esterase content of the serum might be an index of the sympathetic-parasympathetic balance in cases of cardiac irritability, especially in cases of paroxysmal tachycardia of various types.

In a general way it can be stated that the values for this group fall within the limits of variation of the normal. This was found to be true in cases associated with organic cardiac lesions as well as in those in which periodic ectopic rhythms were the only cardiac abnormality. The values can be summarized as follows: 1.80 for a man 32 years old who had mitral stenosis and paroxysmal tachycardia (probably auricular fibrillation); 1.73 for a man 52 years old who had chronic auricular fibrillation associated with arteriosclerotic cardiac disease; 1.88 and 1.57, respectively, for two patients who recently had suffered from attacks of acute coronary thrombosis; 1.78 and 1.79, respectively, for two patients both aged 33 years who had minimal mitral endocarditis associated with considerable anxiety and heart consciousness. Six patients had paroxysmal tachycardia, but no demonstrable evidence of organic heart disease. The values of acetylcholine esterase in these patients were 2.14, 1.89, 1.36, 2.03, 2.31, and 3.31, respectively; all were determined while the cardiac rhythm was normal. Although the value in one was somewhat more than 3.00, which is the upper value of normality, only one such case was found among the 6 studied, and is not considered significant. In the first two cases the opportunity to determine levels of acetylcholine esterase during paroxysmal tachycardia also presented itself; readings were 2.00 and 1.39, respectively (as compared to the levels of 2.14 and 1.89, respectively, when the cardiac rhythm was normal). It is doubtful whether the variations noted are significantly great, but the change was at least in the same direction and the values were lower in the course of paroxysmal tachycardia than in the periods of normal rhythm. Before any definite conclusions could be drawn, however, the concentration of

acetylcholine esterase in serum would have to be determined at more frequent intervals before, during, and after paroxysmal tachycardia.

Pregnancy.—The values for acetylcholine esterase in the serum of 14 women, who either were pregnant at the time or had recently given birth to a child, were determined, and the results are listed in Table II. In two women readings were obtained before and after delivery. It will be noted that while the readings obtained in the course of pregnancy fall within the limits of variation of normal, with two exceptions they are all below the mean found for females (1.72). The mean in the 14 cases of pregnancy was (1.27 ± 0.08), and the difference from the mean for females is significant (0.45 ± 0.11). In one instance in which the occasion presented itself to repeat the determination of the value for acetylcholine esterase in the serum five months post partum, there was a considerable increase over the value obtained during the earlier months of pregnancy (3.02 as compared with 1.31). This finding in association with the rather low values noted during pregnancy prompts further investigation of the behavior of acetylcholine esterase in the serum in relation to pregnancy. Opportunity has not appeared thus far to determine values of acetylcholine esterase in the serum in cases of eclampsia.

TABLE II

VALUES FOR ACETYLCHOLINE ESTERASE DURING AND AFTER TERMINATION OF PREGNANCY

AGE OF PATIENT (YR.)	ANTE PARTUM		POST PARTUM	
	ACETYLCHOLINE ESTERASE (C.C.)*	MONTH OF PREGNANCY	ACETYLCHOLINE ESTERASE (C.C.)*	TIME AFTER DELIVERY
27	1.00	3		
26	1.37	3		
28	1.26	5		
34	1.24	3		
22	0.96	4		
21	1.11	5		
33	1.30	7		
21	1.24	7		
25	0.77	6		
32	1.74	8		
27			0.75	2 days
17†	1.06	5		
18†	2.01	8½		
29	1.31	3	3.02	5 mo.
26	1.39	1 day ante partum	1.51	1 day

*Cubic centimeter of 0.01 normal solution of sodium hydroxide.
†Two different pregnancies in same case.

Disease of Liver and Biliary Tract.—The values in 3 patients with catarrhal jaundice varied from 1.31 to 1.90. The values in 3 patients with stricture of the common bile duct and biliary cirrhosis were low; they were 1.03, 0.74, and 0.92. The values in 2 patients with neoplastic disease of the liver also were low, that is, 1.37 and 0.63. The values in 7 patients with marked hepatitis (surgical description) were low; they were 1.05, 0.69, 1.12, 1.54, 1.01, 0.62, and 0.97, and averaged 1.00. The two values of 0.62 and 0.69 were less than the lower limit of the range of normality. The values in 5 patients with compensated cirrhosis of the liver were just as low as in the group of cases of hepatitis; they were 0.82, 0.89, 0.99, 1.04, and 1.14, and averaged 0.98. It is to be noted

that the values were in the lowest range of values for normal persons, and in one case was slightly less than any of the normal values. The values in 7 patients with decompensated atrophic cirrhosis of the liver were very low; they were 0.22, 0.53, 0.50, 0.62, 0.46, 0.41, and 0.41, and averaged 0.43. In every case the values were much lower than the lowest values for normal persons and much lower than those encountered in severe hepatitis, biliary cirrhosis, and compensated cirrhosis. It is clear that the more extensive and severe the hepatic damage, the lower the values for acetylcholine esterase.

Pancreatic Disease.—The values for acetylcholine esterase were 1.46, 1.49, and 1.57 respectively, in 3 patients with subacute pancreatitis, and 1.23, 0.95, 0.18, 1.68, and 1.55 in 5 patients with chronic pancreatitis with recurring exacerbations. The values with the exception of one were within the range found in normal persons; in the patient in whom the value was 0.18, extensive pancreatic damage without recognizable hepatic disease was found at surgical exploration; as the condition of the patient improved, however, the value for acetylcholine esterase gradually increased to 1.03 fourteen days later. This one low value suggests that pancreatic disease may depress values for acetylcholine esterase, but no conclusions can be drawn.

The values for acetylcholine esterase in 8 patients with carcinoma of the head, and one of the body of the pancreas ranged from 0.77 to 1.47, and averaged 1.18. Only one value was below the normal range; in this patient there was extensive hepatic metastasis. In another patient the initial value was 1.43, but in ten days, as the condition became much worse, the value decreased to 0.61. When the degree of hepatic damage is considered in these cases, it is interesting that the values for acetylcholine esterase were as high as they were.

EFFECT OF VARIOUS DRUGS ON VALUES FOR ACETYLCHOLINE ESTERASE IN THE SERUM

While no systematic attempt was made to study the effect of various drugs on values for acetylcholine esterase, certain observations were recorded. Values were determined in a case of exophthalmic goiter and myasthenia gravis before the administration of 15 mg. of prostigmine orally and fifteen minutes after administration. The values decreased from 1.57 to 1.15. Values before and after two weeks of histaminase therapy in a patient with erythromelalgia were 2.45 and 2.55, respectively. Values before and ten minutes after intravenous administration of 50 mg. of thiamine chloride were 1.55 and 1.59, respectively. Values before and at the height of the pancreatic response to the administration of secretin intravenously were 1.73 and 1.69, respectively. Histaminase, thiamine chloride, and secretin had no effect in these cases on the values for acetylcholine esterase. On the other hand, prostigmine apparently decreased the values transiently.

VALUES IN PANCREATIC JUICE

The values for acetylcholine esterase in pancreatic juice obtained by duodenal tube were 0.14 and 0.0, respectively, ten and twenty minutes after stimulation with purified secretin, and that in pancreatic juice obtained from a human being, who had an external pancreatic fistula, was 0.03.

COMMENT

Our limited group of cases does not permit an elaborate statistical study, but from these data it seems safe to assume that in the presence of severe hepatic damage the acetylcholine esterase activity of the blood serum is decreased. We have observed that not all diseases of the liver or biliary tract are associated necessarily with a low value of acetylcholine esterase, but rather that the low value of acetylcholine esterase is usually a rough index of the amount of damage to the hepatic parenchyma. These findings are in close agreement with the results of McArdle.[8] In 79 per cent of his 71 cases of disease of the liver, acetylcholine esterase values were below the range for normal adults. In 23 cases of cirrhosis of the liver the mean value was less than half of that of normal adults. There seems to be no satisfactory explanation for the decrease of acetylcholine esterase activity in disease of the liver. It has been suggested that bile salts in vitro will inhibit esterase activity,[7, 10] but McArdle's work does not support this hypothesis.

A reduction in values during pregnancy also may be assumed, but a suitable explanation has not yet been advanced.

Our observations in man do not coincide with the observation of Ginsberg and his associates,[11] who found the pancreatic juice of a dog a rich source of acetylcholine esterase. Nor did the parenteral administration of 50 mg. of thiamine chloride to man have any effect on the esterase activity, although an inhibiting effect has been noted in the serum of horses and rats.[12]

SUMMARY

The values of acetylcholine esterase in the blood serum of normal persons and persons with various diseases were determined. For 47 normal men the mean value was 2.06 ± 0.07 and the standard deviation was 0.48. For 38 normal women the mean was 1.72 ± 0.08 and the standard deviation was 0.50. For all the normal persons taken together, the mean was 1.90 and the standard deviation was 0.53. Accepting ± 2 times the standard deviation as a reasonable range for normality, we set the range for normal persons as from 0.85 to 3.0.

Values for acetylcholine esterase in the blood serum were within the range of normal in patients with chronic nervous exhaustion, neurosis, diabetes mellitus, epilepsy, obesity, urticaria, angioneurotic edema, duodenal ulcer, syphilis of the central nervous system, sprue, postural hypotension, arthritis, hypertension of all degrees of severity, heart disease, both organic and that in which periodic ectopic rhythms were the only abnormality, and disease of the biliary tract without severe disease of the liver. Values for acetylcholine esterase were significantly low in pregnancy and in instances of severe hepatic damage. A loose parallelism existed between the values for acetylcholine esterase in the serum and the severity of liver disease. The lowest values were obtained in those patients in whom damage to the liver was most severe (decompensated portal cirrhosis).

Values for acetylcholine esterase in patients with pancreatic disease were, with one exception, within the range of normal. The single low value was so low that the possibility of disease of the pancreas lowering values must be entertained.

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LABORATORY METHODS

GENERAL

A "TRANSPARENT CHOCOLATE AGAR" FOR THE PRIMARY ISOLATION OF THE NEISSERIA AND HEMOGLOBINOPHILES*

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IT IS customary for the personnel of a bacteriologic laboratory to have available, in addition to blood agar, special media for the cultivation of such fastidious organisms as the gonococcus, meningococcus, and influenza bacillus. Many laboratories often employ different media for the isolation of each of the microorganisms mentioned. This imposes a considerable burden on the personnel and on the facilities of the laboratory.

Ever since Cohen and Fitzgerald[1] introduced a cooked blood agar (chocolate agar) and emphasized its usefulness for isolating *Hemophilus influenzae*, many investigators have employed such a medium for the cultivation of both the Neisseria and the hemoglobinophiles. McLeod and his co-workers,[2] Leahy and Carpenter,[3] Reitzel and Kohl[4] reported satisfactory results with various "chocolate agars" for the primary isolation of the gonococcus. Janeway,[5] commenting on medical progress in bacteriology, recommends fresh "chocolate agar" plates or slants for the cultivation of the influenza bacillus and meningococcus from spinal fluid.

Recently, bacto proteose No. 3 agar (Difco[6]), enriched with either bacto hemoglobin, 5 per cent heated defibrinated or citrated blood, has been recommended for the cultivation of the Neisseria. The medium has been used successfully by Carpenter,[7] and is highly recommended by Herrold,[8] and Sulkin and Gottlieb.[9]

While the efficiency of the proteose No. 3 "chocolate agar" has been established, its opacity presents a disadvantage. Leahy and Carpenter,[3] referring to the cultivation of the gonococcus, are of the opinion that the direct inspection of a "chocolate agar" plate is almost valueless when obscured by a luxuriant growth of other organisms. While the application of the oxidase reaction[2] decreases the difficulty due to the opacity of the medium, the promiscuous use of this test has been shown by Cohn and Kruger[10] to have certain disadvantages. The isolation of small numbers of influenza bacilli, however, from a "chocolate agar" plate presents greater difficulty than is encountered in the isolation of the gonococcus and meningococcus, since no reaction similar to the oxidase test is available.

*From the Pathological Laboratory, Division of Bacteriology, Queens General Hospital, Jamaica.

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Proteose No. 3 "chocolate agar" possesses the qualities of an ideal medium, namely: stability of ingredients, a high degree of efficiency, and simplicity of composition and preparation. A medium of similar composition which would maintain all the above qualities and also be transparent seems very desirable.

It is conventional in the preparation of a "chocolate agar" to add 5 or 10 c.c. of sterile defibrinated or citrated blood to an agar base at 80° to 100° C., followed by pouring the so-called "chocolatized" ingredients into a Petri dish. It was found that by centrifuging the heated, coagulated blood, proteose No. 3 agar combination at a moderate speed, a supernatant fluid resulted which when poured into Petri dishes was transparent. The opaque sediment which is discarded never exceeds 2 c.c. for each 21 c.c. of the centrifuged mixture. The proteose No. 3 agar base was further enriched by the addition of a small amount of soluble starch (0.1 per cent) which aided in the production of larger colonies without overgrowth by other organisms.

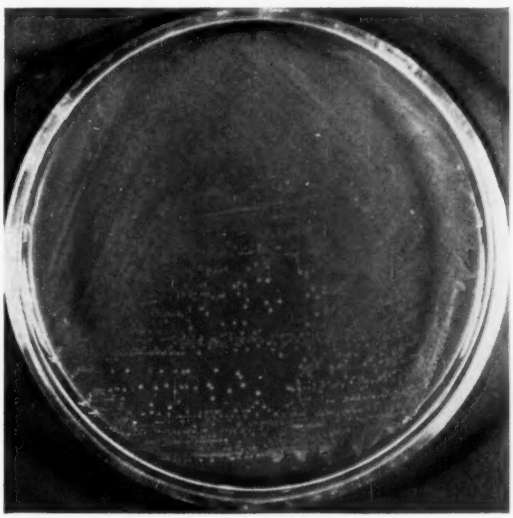

Fig. 1.—Eighteen-hour, primary growth of *Hemophilus influenzae* on "transparent chocolate agar."

The medium in its final form was found to possess all the previously enumerated suitable qualities. Its simplicity of preparation and its ability to support luxuriant primary growth of the meningococcus, gonococcus, and influenza bacillus make it a very suitable medium for the routine of a clinical bacteriologic laboratory. In addition, its transparency presents an added advantage, for the colonies sought can be readily distinguished in mixed cultures.

Over a period of several months we have found this medium a satisfactory addition to the laboratory for the following multiple uses:

1. Routine cultures of spinal fluid and blood from cases of influenzal and epidemic meningitis.

2. Determination of the presence of meningococcus carriers, culturing the material from the nasopharynx.

3. Routine cultures of exudate from all types of gonococcal infections.

4. The isolation of the influenza bacillus from the nose, throat, and sputum.
5. Isolation of *Hemophilus conjunctivitidis* from cases of conjunctivitis.

We hope to analyze the results of this work in a future publication.

PREPARATION

Stock agar base. Suspend 1 Gm. of soluble starch in 1,000 c.c. of cold distilled water. Mix well. Add 45 Gm. of proteose No. 3 agar (Difco), and mix. Boil for one to two minutes to dissolve the agar and starch. Distribute to test tubes in 20 or 40 c.c. amounts, or as desired. Sterilize in autoclave for twenty minutes at 15 pounds pressure.

Fig. 2.

Fig. 3.

Fig. 2.—Eighteen-hour, aerobic, primary growth of the meningococcus.

Fig. 3.—Thirty-six-hour primary growth of the gonococcus in 10 per cent carbon dioxide atmosphere (oxidase positive).

Preparation of final medium. For every 20 c.c. of melted stock agar base, which has been cooled to about 50° C., add 1 c.c. of sterile defibrinated or citrated blood (human blood is used in our laboratory). Mix well. Heat mixture in water bath to 80° C. to 85° C. and allow to remain at the temperature until the blood begins to coagulate.

Centrifuge at 2,000 r.p.m. for one to two minutes.

Pour the supernatant fluid into a Petri dish or prepare slants.

DESCRIPTION OF COLONIES

In eighteen to twenty-four hours colonies of the influenza bacillus are colorless, transparent, and vary in diameter from 1 to 3 mm. On slants growth is luxuriant in eighteen hours, some strains producing colonies which are 2 to 3 mm. in diameter (Fig. 1).

In eighteen to twenty-four hours meningococci appear as circular, moist, shiny colonies, which are colorless by transmitted light. By reflected light the colonies are grayish and semiopaque. The size of the colonies is usually from 2 to 4 mm. in diameter (Fig. 2).

In twenty-four to thirty-six hours colonies of the gonococcus are semiopaque, colorless, opalescent, and have undulated margins. An overcrowded plate may reveal colonies which are 0.5 to 1 mm. in diameter. On a plate which allows ample growing space the colonies may reach 3 mm. in diameter (Fig. 3).

CONCLUSIONS

1. A modification of "chocolate agar," which is stable, simple to prepare, and yields a transparent medium, is presented.

2. Its ability to support initial growth of the influenza bacillus, meningococcus, and gonococcus make this medium a valuable addition to the clinical bacteriologic laboratory.

3. It eliminates the need for several separate media for the cultivation of the Neisseria and the hemoglobinophiles.

We wish to express our appreciation to Dr. Alfred Angrist, pathologist of Queens General Hospital, for his generous advice and cooperation.

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TITRATION AND STANDARDIZATION OF ANTIGEN FOR THE EAGLE FLOCCULATION TEST*

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ANTIGENS manufactured in this laboratory for the Eagle flocculation test have repeatedly failed to equal in reactivity standard antigen received from the test originator. Since a method for antigen adjustment has not been available, only two alternatives were open. Many antigen extracts could be made with the hope of finding one of suitable reactivity or a substandard antigen might be used in the test. Neither of these choices seemed to present an acceptable solution, and, for this reason, an investigation of the methods that might be used for the adjustment of this antigen was undertaken.

It is generally agreed that the reactivity of an antigen is influenced by changes in the sterol-lipoid ratio. Since the Eagle antigen closely approaches saturation with sterols, the possibility of increasing this component is limited. In a preliminary study, however, it has been found that an increase in the lipoid concentration approximating 100 per cent is possible without necessitating a reduction in the specified sterol concentration. It was also shown that, although some antigen preparations could be adjusted to a satisfactory level through an increase in lipoid concentration, others could not be rendered satisfactory by this procedure alone.

Eagle[1] recognized that the electrolyte concentration of the saline diluent may also influence the reactivity level of the antigen, but implied that too great a concentration may have deleterious effects. The results obtained by the combined use of these methods are presented.

Four antigens were prepared from commercial beef heart powder in exact accordance with the published technique of the originator.[2] Sterolization was carried out with Pfanstiehl cholesterol and Difco corn germ sterol. Dilutions of each of these antigens in 0.85 per cent saline (1 part antigen with 2 parts saline) were prepared and stored in the refrigerator for twenty-four hours. For control purposes a similar antigen dilution was prepared from standard antigen that had been obtained from the originator. These stock antigen dilutions were then rediluted with eight parts of 4 per cent saline and tested with serial serum dilutions.

Serum dilutions for the titration of antigens were prepared by diluting previously tested, pooled, positive sera in negative serum, care being exercised to insure adequate mixing. The following dilution schedule was used:

Tube 1.	Positive serum –	3 c.c.	
Tube 2.	Positive serum –	2 c.c.	Plus negative serum – 2 c.c.

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Tube 3.	Positive serum –	1 c.c.	Plus negative serum – 3 c.c.
Tube 4.	Positive serum –	0.5 c.c.	Plus negative serum – 3.5 c.c.
Tube 5.	Serum from tube 2 –	0.5 c.c.	Plus negative serum – 3.5 c.c.
Tube 6.	Serum from tube 3 –	0.5 c.c.	Plus negative serum – 3.5 c.c.
Tube 7.	Serum from tube 4 –	0.5 c.c.	Plus negative serum – 3.5 c.c.
Tube 8.	Serum from tube 5 –	0.5 c.c.	Plus negative serum – 3.5 c.c.
Tube 9.	Negative serum –	3 c.c.	
Tube 10.	Negative serum –	3 c.c.	

The results of the preliminary titration of these four antigens and the control are shown in Table I. As indicated in this table the control or standard antigen gave some degree of positivity in the seventh dilution, whereas the trial preparations gave negative macroscopic and microscopic readings considerably above this level. On this basis the trial antigens were not considered as having standard reactivity.

TABLE I

TUBE	SERUM (0.2 C.C.)	0.1 C.C. OF ANTIGEN DILUTION (1 PART STOCK ANTIGEN DILUTED + 8 PARTS 4.0% SALINE)				
		ANTIGEN A	ANTIGEN B	ANTIGEN C	ANTIGEN D	STANDARD ANTIGEN
1	Positive	POS.-pos.	POS.-pos.	POS.-pos.	POS.-pos.	POS.-pos.
2	Pos. + Neg. (1:2)	POS.-pos.	POS.-pos.	POS.-pos.	POS.-pos.	POS.-pos.
3	Pos. + Neg. (1:4)	POS.-pos.	POS.-pos.	POS.-pos.	POS.-pos.	POS.-pos.
4	Pos. + Neg. (1:8)	DBT.-pos.	POS.-pos.	NEG.-dbt.	DBT.-pos.	POS.-pos.
5	Pos. + Neg. (1:16)	NEG.-neg.	NEG.-dbt.	NEG.-neg.	NEG.-neg.	POS.-pos.
6	Pos. + Neg. (1:32)	NEG.-neg.	NEG.-neg.	NEG.-neg.	NEG.-neg.	DBT.-pos.
7	Pos. + Neg. (1:64)	NEG.-neg.	NEG.-neg.	NEG.-neg.	NEG.-neg.	NEG.-dbt.
8	Pos. + Neg. (1:128)	NEG.-neg.	NEG.-neg.	NEG.-neg.	NEG.-neg.	NEG.-neg.
9	Negative	NEG.-neg.	NEG.-neg.	NEG.-neg.	NEG.-neg.	NEG.-neg.
10	Negative	NEG.-neg.	NEG.-neg.	NEG.-neg.	NEG.-neg.	NEG.-neg.

All tubes shaken for five minutes on Kahn shaking machine and centrifuged at 2,000 r.p.m. before reading.

POS., DBT., and NEG.—Positive, doubtful, and negative *macroscopic* readings.

Pos., dbt., and neg.—Positive, doubtful, and negative *microscopic* readings.

In an effort to adjust these four antigens to the level of reactivity of the control, the method of increasing lipoid content was utilized. This was accomplished by evaporating alcohol from measured portions of the unsterolized antigens until reductions to three-fourth and one-half the original volumes were obtained. Thus, when 100 ml. of antigen were reduced to 75 ml., a 33 per cent increase in lipoid content was obtained, and when further reduction to 50 ml. was accomplished, a 100 per cent increase in lipoid content was effected. These concentrated antigens were sterolized and filtered, and stock antigen dilutions were prepared as previously described. After twenty-four hours' refrigeration portions of each of these stock antigen dilutions were rediluted with eight volumes of 4 per cent, 6 per cent, 8 per cent, and 10 per cent salines, and retitrated against standard antigen using the previously described serum dilutions. To eliminate duplication of data only one protocol, considered to be representative of the group, is presented in Table II.

The findings indicate that antigen A was not adjusted to standard reactivity by the use of increased saline concentrations alone. Nor did increased lipoid contents of 33 per cent, or 100 per cent, attain a satisfactory reactivity level when used with 4 per cent saline. However, a reactivity as sensitive as that of the standard antigen was obtained at two points: 33 per cent increased lipoid with 10 per cent saline and 100 per cent increased lipoid with 8 per cent

saline. Antigen with 33 per cent increased lipoid when used with 10 per cent saline, although sufficiently reactive with positive sera, gave unsatisfactory results (see Discussion) in the negative serum tests, so the final adjustment chosen for this antigen was 100 per cent increase of lipoids with 8 per cent saline as the secondary diluent. Similar titrations of the other three antigens, designated 100 per cent increase in lipoids with 8 per cent saline, in one instance, and 100 per cent increase in lipoids with 6 per cent saline, in the other two, as the proper adjustment end points. All four of these antigens, after being adjusted in accordance with the titration results, were found to be of standard reactivity as compared against standard antigen when tested with positive and negative sera.

TABLE II

TUBE	SERUM	ANTIGEN A				ANTIGEN A LIPOID CONTENT INCREASED 33%				ANTIGEN A LIPOID CONTENT INCREASED 100%				STANDARD ANTIGEN
		SALINE CONTENT OF SECONDARY DILUENT												
		4%	6%	8%	10%	4%	6%	8%	10%	4%	6%	8%	10%	4%
1	Positive	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p
2	Pos. + Neg. (1:2)	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p
3	Pos. + Neg. (1:4)	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p
4	Pos. + Neg. (1:8)	D-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p	P-p
5	Pos. + Neg. (1:16)	N-n	N-n	D-p	D-p	N-d	P-p	P-p	P-p	P-d	P-p	P-p	P-p	P-p
6	Pos. + Neg. (1:32)	N-n	N-n	N-n	N-d	N-n	N-d	N-p	D-p	N-d	D-p	D-p	P-p	D-p
7	Pos. + Neg. (1:64)	N-n	N-n	N-n	N*-d	N-n	N-n	N-n	N*-d	N-n	N-n	N-d	P-p	N-d
8	Pos. + Neg. (1:128)	N-n	N-n	N-n	N*-d	N-n	N-n	N-n	N*-d	N-n	N-n	N-n	N-d	N-n
9	Negative	N-n	N-n	N-n	N*-d	N-n	N-n	N-n	N*-d	N-n	N-n	N-n	N-n	N-n
10	Negative	N-n	N-n	N-n	N*-d	N-n	N-n	N-n	N*-d	N-n	N-n	N-n	N-n	N-n

All tubes shaken five minutes on Kahn shaking machine and centrifuged at 2,000 r.p.m. before reading.

Either 0.4 c.c. of serum plus 0.2 c.c. of antigen dilution, or 0.2 c.c. of serum with 0.1 c.c. of antigen dilution may be used.

P, D, N—Positive, doubtful, and negative *macroscopic* readings.

p, d, n—Positive, doubtful, and negative *microscopic* readings.

*Swirl appearance missing.

DISCUSSION

The titration results presented in Table II show that the unadjusted antigen and the antigen containing 33 per cent increased lipoids gave rough microscopic pictures with negative sera when the 10 per cent saline diluent was employed. In these tubes the typical negative swirl was missing, the gross appearance being more chalky than usual. For this reason, these adjustment end points could not be considered suitable, even though a satisfactory reactivity with positive serum was attained.

These microscopic readings are considered an essential part of the titration, since they allow acute differential observations to be made. This is especially necessary in the negative serum tests so that an antigen adjustment can be made that will not give rough negative microscopic pictures. However, if two or more

adjustments are found to be equally good, the lesser lipoid content with the lower saline concentration is probably to be preferred.

The level of reactivity that is now considered satisfactory for any test antigen is chosen by the author of that particular test. If, for some reason, the test author decides to raise or lower the test sensitivity level by changing the antigen reactivity, readjustments of antigen and saline can be made that will reproduce the desired result. For this reason, it is necessary that a standard antigen be available for comparative antigen titrations.

However, since the two antigen adjustments used are quantitative in nature, it is not presumed that sufficient correction can be obtained by these methods alone to compensate for any qualitative changes that may occur in some beef heart powder. Such unsatisfactory beef heart powder has not been encountered in this laboratory to date.

SUMMARY

Four Eagle flocculation antigens prepared from commercial beef heart powders were found to have varied reactivity, none of which were equal in reactivity to standard antigen obtained from Eagle.

A titration method for the comparison of antigen reactivity and a procedure which will allow the accurate adjustment of Eagle flocculation antigen is described.

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A STUDY OF RECENTLY ISOLATED STRAINS OF STAPHYLOCOCCI AND THEIR ABILITY TO COAGULATE HUMAN PLASMA*

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MOST of the literature published the last few years on the various laboratory methods used to determine pathogenicity of staphylococci has not stressed the value of the plasma-coagulase reaction. Chapman[1] and his co-workers discussed the value of this test as compared to the hemolysis of blood, the pathogenicity for rabbits, and the agglutination test. These investigators, however, did not correlate the ability to coagulate plasma with the source of the strain. Their observations were made on five thousand strains of staphylococci with no classification as to their source. Fisher,[2] reporting on thirty strains isolated from human staphylococci lesions, found that all exhibited the ability to coagulate plasma.

The present study was made on 65 freshly isolated strains of staphylococci. Nineteen of these strains were isolated from patients with clinical manifestations

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†Cultures obtained from the Department of Bacteriology and Parasitology at the University of Chicago through the courtesy of Dr. G. M. Dack.

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of staphylococcus infection. Thirteen were collected from samples of raw milk, 24 were isolated from the air as contaminants, and nine were strains recovered from outbreaks of food poisoning in which the toxicity had been experimentally demonstrated.† All the strains of staphylococci used in this study fermented lactose and coagulated milk with acid production.

To determine chromogenicity, transplants were made on 1 per cent starch infusion agar so that well-isolated colonies were obtained. The plates were incubated at room temperature (27° to 30° C.) for three days before pigment formation was determined. Toward the end of this study, however, it was found that pigment production could best be observed by growing the staphylococci on milk agar plates. This medium was prepared by adding, aseptically, from a can of evaporated milk,* 10 c.c. of milk to 90 c.c. of melted agar. The agar should be cooled to 50° C. before the milk is added. Pigment production was easily observed on this medium after the streaked plates had been incubated at 30° C. for three days.

To determine hemolytic activity, the staphylococci were cultured on extract agar plates in which 5 per cent defibrinated human blood had been incorporated. The observations were less variable on repeated tests with the use of human blood than with the use of rabbit blood.

The ability to coagulate plasma was demonstrated by inoculating 0.1 c.c. of a twenty-four-hour broth culture of staphylococci into 3 c.c. of diluted human plasma. The diluted plasma was prepared by adding one part of sterile physiologic salt solution to three parts of sterile citrated human plasma. The subcultures were incubated at 37° C. for eighteen hours before readings were recorded.

TABLE I

STRAINS ISOLATED	SOURCE OF STRAINS	NUMBER PRODUCING PIGMENT	NUMBER HEMOLIZING BLOOD	NUMBER COAGULATING PLASMA
19	Clinical lesions	16	16	19
9	Food poison	8	8	9
13	Raw milk	7	7	7
24	Air and dust	6	1	1
65	All sources	37	32	36

Table I shows the results obtained from this study. Thirty-six strains were capable of coagulating plasma. All 19 strains isolated from patients with clinical manifestations of staphylococci infection coagulated the diluted plasma, while only one of the 21 strains isolated from the air and dust showed this characteristic. Another interesting observation was that the food poisoning strains of proved toxicity were all able to coagulate plasma. This reaction on further study with food poisoning staphylococci may prove to be valuable as an exclusion test in the epidemiologic study of such outbreaks. Only three of the 36 strains capable of coagulating plasma were the "albus" variety; one of these was isolated from milk of a cow with mastitis; another from a patient with an infected mammary gland, and the third was a food poisoning strain.

*The evaporated milk used in these experiments was prepared by the Carnation Milk Company.

A number of samples of raw milk were cultured before thirteen strains of staphylococci were isolated. There is a chance that these were more or less saprophytic strains contaminating the milk during its production and distribution. However, two of them were isolated from the milk of a cow with definite symptoms of mastitis. Both of these strains coagulated plasma. It is possible that the other five coagulating strains of staphylococci recovered from raw milk might have been from cows with unrecognized symptoms of mastitis.

All 65 strains of staphylococci were retested for their ability to coagulate diluted human plasma after having been subcultured on extract agar for twelve months. The results obtained showed complete agreement in all cases with the readings made when the staphylococci were first isolated. Blair,[3] reporting on the stability of the plasma-coagulase reaction, recorded a 94 per cent agreement on the 102 strains tested between the original and the subsequent tests made two and one-half years later.

CONCLUSIONS

The ability of staphylococci to coagulate diluted human plasma is definitely correlated with its pathogenicity for man.

The ability to coagulate human plasma is apparently a stable characteristic of a strain of staphylococci.

The ability to coagulate plasma may prove to be valuable upon further study as an exclusion test in the epidemiologic study of staphylococci food poisoning outbreaks.

Milk agar prepared by the use of commercially manufactured evaporated milk is an excellent medium for determining chromogenicity of staphylococci.

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THE MEASUREMENT OF FORMALDEHYDE FIXATION*

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THE fixation of small protein structures by formaldehyde is effected by maceration in diluted formalin with or without suitable addition agents. In embalming, a similarly constituted solution is injected arterially. As the formaldehyde diffuses inward, it unites principally with the terminal NH_2 groups of the protein structures, causing them to set to a more or less rigid gel. The degree of setting, hardness, or fixation of the preserved tissue is a matter of considerable importance, since it is usually desirable to maintain, as nearly as possible, the original consistency and appearance of the tissue. This is especially true in the case of dissecting material and in the embalming of cadavers. Formaldehyde is capable of greatly changing the consistency and gross appearance of tissues when used alone in aqueous solution, and it is, therefore, customary to add to it various other compounds designed to control or to direct its action. These concern mostly preservation and color. The various modifications of the Kaiserling process[1] constitute a case in point.

We have made quantitative studies of many of the physical and chemical factors involved in formaldehyde preservation under a grant from the Minnesota State Board of Health. The present report concerns the quantitative measurement of the hardness of formaldehyde-treated protein structures.

Since no commercial device was available for measuring hardness or rigidity of tissues on a numerical scale, we devised one. This instrument was a modification of one previously used by one of the authors[2] in other biological studies of physical and chemical factors in biology. In effect it measures the distance through which the surface of the specimen is depressed when a fixed force is applied to a small area of it.

DESCRIPTION OF THE METER

Our meter was adapted from a 3-inch vacuum gauge and is shown in Fig. 1. The flexible ribbon and pipe connection were removed, and a tubular brass sleeve *S*, ¾ inch by 1½ inches, carrying three short sturdy prongs *P* at its lower end, was fitted to the case. A ⅛-inch brass rod *R* was then fitted to operate smoothly and without extra play within the sleeve. The upper end of the rod was attached to the lever system *L*, operating the pointer, and the lower rounded end of the rod was cut to such a length that it projected ⅛ inch beyond the plane of the sleeve prongs when the pointer was at zero. A normalized spring was then fitted to retain the rod in the extended position. The strength of the

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spring was such that a weight of 120 Gm. was required to displace the end of the rod into the plane of the three-sleeve prongs.

Similar devices may be constructed from small pressure gauges, opticians' diopter gauges, or dial reading auto tire gauges. It is essential to have a scale with large well-marked divisions, so that the readings can be taken at a single glance, and the stiffness of the spring must be suited to the range of pliability of the specimens to be measured. The spring which we made and used allowed a scale reading of only one division when the meter was applied to soft living tissues or fresh hog kidneys, and a reading of about 25 on embalmed dissection cadavers.

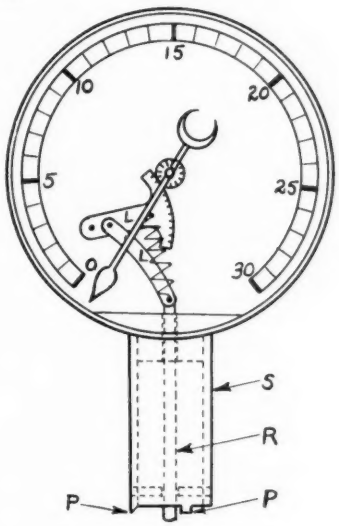

Fig. 1.—Fixation meter.

USE OF METER

In use, the protruding plunger of the meter is brought perpendicularly against the surface of the specimen and pressed carefully against it until the three-sleeve prongs barely touch the surface. The dial reading is then taken. Several readings over representative areas are taken and the results are averaged. Fleshy areas, which are more or less flat, are to be preferred since underlying bony structures affect the readings somewhat. By choosing like anatomic areas, consistent readings are easily obtained. In cadavers the abdomen and ventral surfaces of the thigh and forearm are preferred, since dependent areas are usually somewhat flattened, distorted, and otherwise changed.

APPLICATIONS

In living bodies the changes in muscle tonus during contraction and relaxation may be measured readily. The changes in tissue consistency accompanying the deposition of fat may be measured. The progress of rigor mortis with time is likewise measurable. The softening of structures following incipient putrefaction also may be followed. We have made hundreds of measurements with this meter upon various subjects, including man, in connection with our quantitative studies on the various phases of action of embalming fluid chemicals.

The time course of the so-called "embalming reaction," which occurs during and after the arterial injection of simple formaldehyde solutions, is shown graphically in Fig. 2. The curves show that the fixation begins when injection starts, increasing very rapidly, but not reaching a maximum until forty-eight hours or more have elapsed. So far as we are aware, no measurements like these have ever been reported, nor has any reference ever been made regarding a

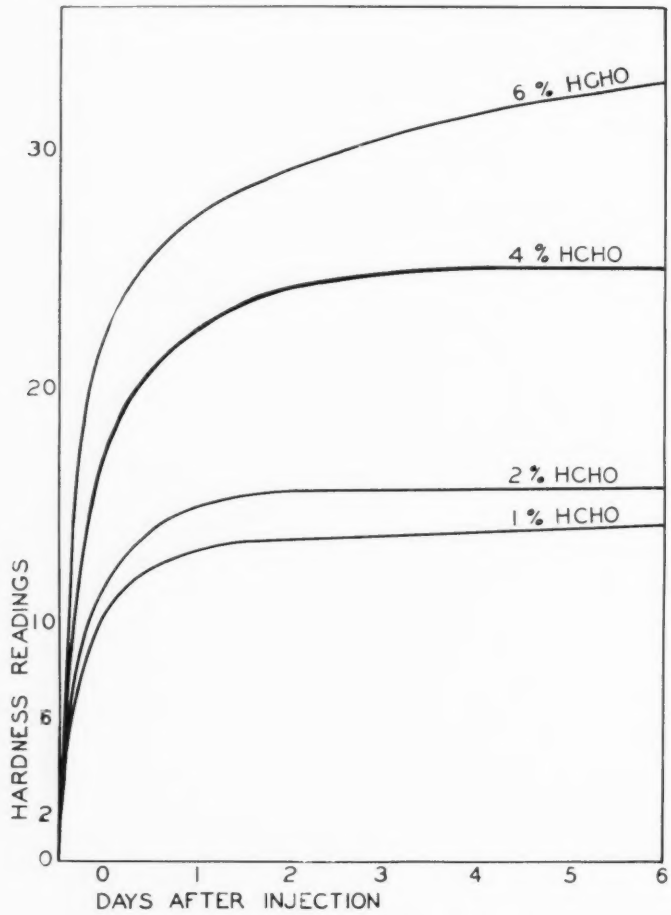

Fig. 2.—Time course of fixation reaction.

delayed fixing or embalming reaction. We believe that such postinjection hardness increases are due to a delayed action in which formaldehyde solutions of decreasing strength diffuse slowly into contact with still unattacked protein structures. Finally, the hardness becomes stabilized, but it may later decrease if the amount of formaldehyde used is so small that putrefaction begins.

The effect of increasing concentration of formaldehyde upon the hardness of tissue is also shown in Fig. 2. The curves represent the first published data upon the subject. All the curves show the time lag already mentioned. It is interesting to note that fixation does not increase directly with concentration. This suggests an adsorption phenomenon, but it may also be due to the "walling-

off'' of the solution by local solid areas which interfere with the diffusion of the aldehyde. In time the compound may diffuse through, but then there is less of it. As a result the hardness is less than it would otherwise be. We are continuing the study of this point.

CONCLUSIONS

1. A simple portable meter has been devised for measuring the hardness of tissues, organs, or cadavers on a numerical scale.

2. Changes in the hardness of tissues under varying conditions and modes of treatment can be easily measured.

3. Only a few minutes of experience is needed in order to obtain consistent readings on hardness.

4. The use of the meter does not deface the anatomic structures in any way.

5. The instrument has proved useful in obtaining measurements regarding the synergistic effects of various compounds used in embalming fluids.

6. No numerical data of this kind are to be found in previous literature on the subject.

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A SIMPLE AND ACCURATE METHOD OF COUNTING SPERMATOZOA*

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THERE are a number of various techniques in present-day general use that are employed in counting spermatozoa in a routine semen analysis. All these methods are complicated either by the use of blood counting pipettes or by the special diluting fluids. The method of Macomber and Saunders[1] involves the use of a blood-counting pipette (1:20 dilution) and a sodium bicarbonate-formalin solution (5 per cent sodium bicarbonate and 1 per cent formalin). The method of Hotchkiss[2] utilizes a diluent consisting of a solution of saturated sodium bicarbonate together with 1 per cent phenol. The ''bulk'' method of Belding[3] employs larger amounts of semen for dilution, but also makes use of the special Macomber-Saunders diluting fluid.

I use a much simpler method which obviates the necessity of pipettes, blood-counting pipettes, and special diluting fluids. This method is based on a recent report[4] which showed that plain, ordinary tap water is one of the most drastic

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spermicides known. The dilution with water serves not only to dilute the sperm cells so that they may be easily counted in a Neubauer blood-counting chamber, but it also causes immediate cessation of motion of the sperms facilitating the count. The use of tap water as the diluent is especially useful to those who have neither the time nor the facilities to prepare special solutions for an occasional semen analysis.

TECHNIQUE

One cubic centimeter of well-mixed whole semen is placed in an ordinary test tube, graduated at 20 c.c. Then ordinary cold tap water is added to the tube up to the 20 c.c. mark.

This simple technique has two distinct advantages: First, by simply adding water all the spermatozoa are killed at once; second, the use of a whole cubic centimeter of semen diluted 1:20 diminishes the error which crops up when using the small amounts of semen usually utilized in the blood-counting pipette technique.

The sperms are counted in the same fashion as are white blood cells, and the final count is arrived at in the following manner: After 1 sq. mm. is counted and rechecked (the sixteen small squares in one corner of the chamber), the number obtained is multiplied by 10 to constitute the number of sperms found in 1 c.mm. This is multiplied by 1,000 to find the number in 1 c.c. of diluted semen. To arrive at the number contained in 1 c.c. of whole undiluted semen, it is now necessary to multiply once more by 20 (the dilution used).

The above multiplication may be simplified by taking the number found in 1 sq. mm. and adding five zeros to it and multiplying by two.

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THE USE OF THIOGLYCOLLATE MEDIUM IN DIAGNOSTIC BACTERIOLOGY*

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THE use of sodium thioglycollate in infusion medium was described by Brewer in 1940. This medium was described as a fluid medium in which anaerobes could be cultivated without the use of special fermentation tubes or other devices for excluding air. It was also pointed out that the thioglycollate inactivates most of the mercurials commonly used as preservatives in biologic products and is, therefore, useful in testing the sterility of such products. Marshall, Gunnison, and Luxen (1940) have confirmed its usefulness for such purposes. It has been found that this medium is well adapted for the growth of aerobic and micro-aerophilic types of bacteria as well. We have confirmed Brewer's reports and have made extensive use of this medium in routine bacteriologic examinations. After some time we observed that the results obtained by the use of this medium seemed to be better than those obtained with certain others in routine use. A survey of these results revealed the superiority of this medium for certain purposes, particularly in the culturing of specimens of blood and lochia.

This report includes examination of 69 consecutive blood specimens and 93 consecutive uterine specimens. Seven blood cultures were found positive by one or all of the methods used, and 82 uterine specimens were positive by one or more methods.

TECHNIQUE

Blood specimens were, as a rule, planted at the bedside, but if this was not possible, the material was taken directly to the laboratory and placed into the various media within fifteen minutes of the time it was taken from the patient. Five to 10 c.c. of blood were plated directly into 50 c.c. of infusion broth containing dextrose. Duplicate plates were prepared for aerobic and anaerobic incubation, using 1.0 c.c. of the blood specimen in each plate. One cubic centimeter and 2.0 c.c., respectively, were added to tubes of thioglycollate medium. All cultures were incubated at 37° C. and inspected daily for ten days unless growth appeared earlier.

Uterine specimens were brought to the laboratory in Little's tubes wrapped in sterile towels. The contents were transferred to a tube of infusion broth, and inoculations of the various media were made by adding one or more drops of the material to each, depending upon the amount of lochia present. Blood agar plates for aerobic and anaerobic incubation, infusion broth, and thioglycollate medium were used. All media were incubated at 37° C. for ten days before being discarded as sterile, and all were examined daily. When growth appeared

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in broth or in thioglycollate medium, blood agar plates were made to obtain isolated colonies to be fished and studied in pure culture.

It should be noted that in many instances specimens were taken from patients being treated with one of the various sulfonamide compounds. While it has not been our experience (unpublished experiments) that the addition of such substances as p-aminobenzoic acid enhanced the ability of an infusion medium to produce positive blood cultures in such cases, the results that follow might indicate that if such compounds are useful for this purpose, they are to be found naturally in thioglycollate medium.

RESULTS

Of the 69 blood specimens cultured, growth was obtained in only eight. No growth occurred in blood agar either aerobically or anaerobically in ten days. In four cases growth occurred in broth inoculated with 5 to 10 c.c. of the specimen, while growth was obtained in thioglycollate medium in seven cases. In only one instance did growth occur in broth when it did not appear in thioglycollate medium, yet in four cases growth was obtained in thioglycollate medium when it did not appear in broth, although the broth invariably contained from five to ten times as much inoculum. The types of organisms isolated were (1) from broth: alpha streptococci, three times; staphylococci, once; (2) from thioglycollate medium: alpha streptococci, four times; staphylococcus, twice; and pneumococcus, once. Growth appeared in these media as soon as twenty-four hours (in one case), or as late as the seventh day.

Of the 93 uterine specimens cultured, growth occurred on the blood agar plates incubated aerobically in 19 specimens, while those incubated anaerobically gave 16 positive cultures. Broth cultures were positive in 57 instances, and thioglycollate medium gave 82 positive cultures. An analysis of the results obtained with the various media is shown in Table I.

TABLE I

Medium	POSITIVE CULTURES					TOTAL
Thioglycollate medium	9	39	10	7	17	82
Broth	9	39	10	0	0	57
Blood agar plates (Aerobic incubation	9	0	10	0	0	19
Blood agar plates (Anaerobic incubation)	9	0	0	7	0	16

According to these data it would appear that thioglycollate medium has a superiority over broth in a ratio of 82:57; over aerobic blood agar plates the superiority is greater, 82:19. Compared with blood agar incubated anaerobically the ratio is 82:16. Since growth occurred in thioglycollate medium in all cases in which growth occurred in any medium when lochia was cultured, and only failed in one case when blood was being cultured—and in this case it is reasonable to assume that the larger inoculum used in the broth is accountable—it appears that thioglycollate medium has an important use in clinical and diagnostic bacteriology. No doubt some of the superiority lies in the fact that anaerobic as well as aerobic bacteria develop well in this medium.

While this paper was being written, *Neisseria intracellularis* (type III) was isolated from the spinal fluid of a case of meningococcus meningitis simultaneously on thioglycollate medium incubated aerobically and chocolate agar incubated in an atmosphere containing carbon dioxide. Isolation was not accomplished on unheated blood agar.

CONCLUSION

The use of thioglycollate medium by itself, or in conjunction with other media, is recommended in routine diagnostic bacteriology.

Since this paper was sent to the publisher this observation has been repeated in 5 additional cases of meningococcus meningitis. In 4 cases type I meningococcus was found, and in one, type II.

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The thioglycollate medium used in these studies was prepared by the Baltimore Biological Laboratory, Baltimore, Md.

MULTIPLE TISSUE WASHER AND PROCESSING ASSEMBLY*

Combination Apparatus Facilitating the Preparation of Tissues for Paraffin Sections

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CAREFUL manipulation and preparation of relatively large numbers of tissue blocks as a laboratory routine are possible with a minimum of time, labor, and space with a combination multiple tissue washer and processing assemblies described here. This apparatus has been developed and improved as a matter of necessity for saving time, labor, and space in the course of preparing hundreds of tissues for paraffin sections. Careful consideration was given to histologic principles and methods as well as to the factor of minimum handling of the tissues with instruments.

The cost, availability of materials, and construction of the apparatus were given careful consideration. This apparatus can be constructed by a technician or student assistant with materials from the laboratory stockroom, hardware store, department store, woodshop, and the Kimball Glass Company, Vineland, N. J.

The combined apparatus, multiple tissue washer and processing assembly, is composed of the following parts:

1. Tissue washer: a deep, aluminum pan with 27 outlets and rubber tubes with Hoffman clamps (Fig. 1).

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2. Processing assemblies: inverted, open-bottom, screw-cap vials, perforated caps and corks fitting into fruit juice glasses (Fig. 2).
3. Tripod: japanned iron tripod (Fig. 1).
4. Assembly trays: cypress or aluminum (Fig. 1).

The size of the tissue washer, the number of processing assemblies, and the size of the assembly trays will depend chiefly on the requirements of the laboratory. A sufficient number of assemblies should be provided to permit a serial progression of several units of assemblies with tissues through the steps of fixing, washing, dehydrating, clearing, and paraffin infiltrating. In this laboratory, using a tissue washer with 27 tubes, 46 assemblies are used for the first four steps in the procedure and 30 additional assemblies are kept in the paraffin oven for infiltrating the tissues.

The Tissue Washer (Fig. 1).—The multiple tissue washer consists of a deep, 20-gauge, aluminum pudding pan of 6 quarts capacity, perforated with 27 holes jointed to short copper tubes holding 7-inch length rubber tubes provided with Hoffman clamps. The size of the pan and the number of outlets will depend on the requirements of the laboratory. The 27-outlet washer in use in this laboratory has two circular, inner and outer, rows of holes drilled through the bottom of the pan. The outer row contains 18 holes equally spaced. The inner row has 9 holes equally spaced, and each hole is placed opposite one in the outer row. The holes in the inner row should be placed opposite the spaces between the holes in the outer row. The holes are drilled from the outside to the inside. Soft copper tubing, ¼ inch outside diameter is cut into one-inch lengths and forced into the holes. These holes are made slightly smaller to hold the tubes firmly in place until jointed with liquid solder. The height of the tubes inside the pan is about 3/16 inch above the bottom to provide anchorage for the solder. Care is taken that the tubes are perpendicular to the bottom of the pan. Liquid solder is spread around each tube level with its upper end. It is allowed to harden overnight. To further strengthen the support of the tubes in the outer row, which is in a peripheral moat, a ribbon of solder is laid in the moat. Each tube is surrounded by this ribbon of solder. The inner edge of the ribbon reaches the inner rows of tubes, strengthening them likewise. The solder is allowed to harden overnight. The pan is inverted on the tripod, and liquid solder is spread around the copper tubes, strengthening them still more. This tissue washer has been in constant use for more than a year, and not a single tube has loosened or been resoldered. The ribbon of solder in the moat has contracted and drawn away from the wall of the pan, but it has not affected the use of the washer in any way. A rubber tube, 7 inches in length, 1/16 inch wall, ¼ inch bore, is placed over each copper tube, and a Hoffman clamp, ½ inch size, is clamped onto each rubber tube.

This multiple tissue washer possesses the following advantages:

1. It is durable, inexpensive, and easily constructed.
2. It provides a reservoir of water from a single tap for one or 27 assemblies.

3. It provides a constant flow of water, the rate of which is controlled by the Hoffman clamp, and can be regulated to a full stream or to a definite number of drops per minute.
4. It requires only a very small amount of space for operation and storage.

The Processing Assembly (Fig. 2).—The processing assembly consists of an inverted, open-bottom vial, a molded bakelite cap perforated with 5 to 7 holes, a plain 5-ounce fruit juice glass, and two corks, 2½ by ⅝, and 1 by ½ inches in size. The larger cork fits into the glass container, and has a center hole of a size to hold the vial firmly and upright in place, yet permitting easy shifting of its position in the assembly. The smaller cork is used as a stopper in the

Fig. 1.—Photograph of multiple tissue washer and processing assemblies with assembly tray.

top opening of the vial. The vial, size No. 4, 4¼ inches in length, is cut down to a length of about 4 inches. This is accomplished by winding a double loop of a stout cord around the bottom of the vial, and with a see-saw movement of the loose ends of the cord create sufficient friction and heat to cause it to break clean when plunged into ice water. The sharp edges are made smooth by glazing over a Bunsen burner. The molded Bakelite cap is perforated with 5 to 7 holes, the size and number of which will depend on the kind and size of tissues routinely prepared. There should be several perforations in the

cap to permit a free movement of the convexion currents into and out of the lumen of the vial, thus assuring a more perfect interchange of reagents between the tissue and the surrounding medium.

The Tripod (Fig. 1).—The iron tripod is of heavy material. The outside diameter of the ring is 5⅛ inches. The height of the tripod which supports the washer is 9 inches. The diameter of the circle formed by the inner row of tubes on the bottom of the washer should be approximately 7 inches to allow the ring top of the tripod to fit within this circle.

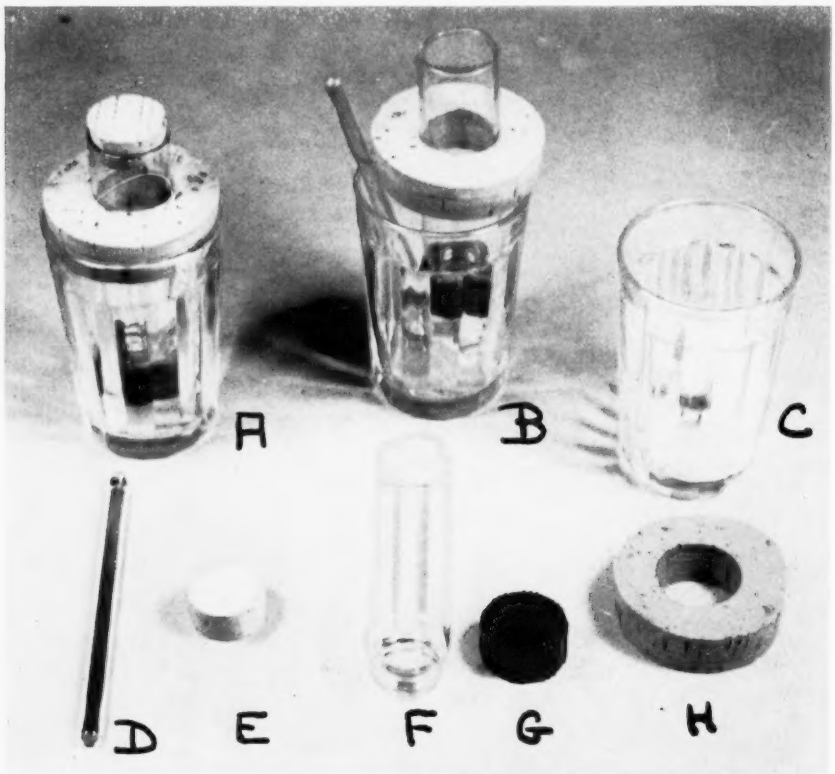

Fig. 2.—Photograph of a processing assembly and its parts. (*A*) Assembly complete for fixing and dehydrating tissues. (*B*) Assembly complete for washing tissues. (*C*) Assembly glass. (*D*) Glass rod. (*E*) Small cork stopper for vial. (*F*) Open-bottom vial. (*G*) Perforated bakelite cap, for vial. (*H*) Large cork with center hole for holding vial.

The Assembly Trays (Fig. 1).—The number of assembly trays is dependent on the size of the washer and the number of assemblies used. If only a few are used, a small tray will be satisfactory, but if 27 or more assemblies are used at one time a large tray will be required. Even though the larger number of assemblies is maintained, the smaller tray will be found very convenient when only a few assemblies are in operation. The small tray is a 22-gauge, aluminum layer-cake pan, 10 inches in diameter with a ¾ inch hole in the wall to serve as an outlet.

A large tray to hold 27 assemblies was designed and constructed from a single piece of cypress, 2 inches in thickness. It was turned on a lathe to form

a tray with an inside diameter of 14 inches, a rim 1 inch high and ½ inch wide, making a total diameter of 15 inches. There are eight 4-inch legs, ¾ inch in thickness, supporting the tray. The tray was designed to be used in a sink.

A second large tray was designed to be used on top of the counter. A wide, shallow "Wear-Ever" aluminum cream pan, No. 401, bottom diameter 14¾ inches, top diameter 16⅝ inches, depth 3¼ inches, was purchased from a hardware store. The pan has a single, large outlet, with a rubber tube attached to carry off the overflow water from the assemblies. This outlet was made by drilling through the wall of the pan a hole ¾ inch in diameter, ¼ inch from the bottom. A piece of copper tubing, ¾ inch in diameter, 1½ inches long, was used for the overflow pipe. At one end of the tube a flange was made by making several longitudinal cuts to a depth of ¼ inch, and pressing the segments outward. The tube was forced through the hole, and the flange was shaped to the curvature of the wall of the pan. The tube was soldered to the wall, inside and outside, of the pan. After hardening, a rubber tube ¾ inch, inside diameter, 1 foot in length, was slipped over the copper overflow pipe.

The durability and practicability of these two large assembly trays have been thoroughly tested and found satisfactory.

ADVANTAGES OF THE PROCESSING ASSEMBLY

The processing assembly is used to carry the tissues through fixation to paraffin imbedding with a minimum handling of the tissues. The principal feature of the assembly is the vial and the perforated bakelite cap, fitting tightly in the center of the hole of the large cork. When this unit is in place, the vial is centered and its distance from the bottom can be changed by shifting its position in the cork. The centering and manipulation of the vial have many advantages.

1. It serves to suspend the tissue in the fixing fluid and permits the penetration of the fixative through all surfaces.
2. It permits thorough washing of the tissue. A rubber tube of the washer is placed inside the vial through the top. A glass rod is placed in the container between the cork and the top edge of the glass.
3. The flow of water through the tube from the reservoir of water is controlled and regulated by the Hoffman clamp.
4. A full stream or only a few drops of water per minute, depending on the character of the tissue, is permitted to flow into the vial.
5. The water circulates in the vial and flows out into the container and over its edge.
6. The glass rod keeps the cork away from one side of the glass, permitting this overflow of water.
7. A continuous flow of fresh water is possible.
8. The loss of the tissue from the vial is prevented as the level of the water inside the vial is below the top opening of the vial.
9. The processing assembly permits the use of calcium oxide and dioxane as dehydrating agents.
10. The vial can be raised above the lumps of calcium oxide.

11. The perforated cap permits the interchange of dioxane and water between the tissue and fluid and the passage of water down to the calcium oxide.
12. The assembly permits dehydration of the tissue with a series of graded alcohols.
13. It permits infiltration of the tissues with paraffin.
14. It permits easy handling of the tissues in the process of imbedding.

A second assembly, kept permanently in the paraffin oven, is used with distinct advantage in this step. The melted paraffin may be kept in the assembly or placed in it prior to transferring the tissue from the first unit. Then the tissue is transferred without handling by inverting the first vial and dropping the tissue into the paraffin in the second vial. The suspension of the tissue in the paraffin allows the paraffin to penetrate into the tissue through all its surfaces, the assembly permits easy handling of the tissue in the process of imbedding. The unit with the paraffin-infiltrated tissue is taken to the imbedding box, and there the tissue is transferred to liquid paraffin in a glass stain jar. The transfer is accomplished by grasping the cork, holding the vial and inverting it, thus avoiding getting hot paraffin on the hands. Occasionally it may be necessary to unscrew the cap to remove the tissue. Placing the tissue in the glass stain jar permits the technician to ascertain its size and shape, thus determining its orientation in the imbedding block. Then the tissue may be transferred to the imbedding block with a perforated spoon.

LIST OF MATERIALS

Capsule vials, screw cap, size No. 4, No. 60957.			
Height 4¼ inches	½ gross	$ 3.75	
Black molded caps	½ gross	1.00	
Purchased from Kimball Glass Company, Vineland, N. J.			

The prices listed are given only to show cost at time of purchase. The following materials may be purchased from any laboratory supply house, hardware store, department store, and woodshop.

Hoffman clamps, each	$ .20	
Tripod	.55	
Rubber tubing, per foot	.08	
Copper tubing, 2½ feet	.30	
Aluminum pudding pan, 20-gauge, 6-quart capacity	1.25	
Aluminum layer-cake pan, 22-gauge, 10-inch diameter	.60	
Liquid metallic solder, tube	.10	
Corks, XXX quality, 2¼ by 2⅛ by ⅝, bag of 100	10.55	
Corks, XXX quality, 1 by ⅞ by ½, bag of 100	2.35	
Assembly tray, cypress	4.50	
Assembly tray, aluminum cream pan, No. 401	3.00	
Glass rod, No. 6, 10.5 ft.	.25	
Approximate cost of tissue washer with 27 outlets:		
Aluminum pan	$ 1.25	
Copper tubing and solder	.75	
Rubber tubing, 16 feet	1.28	
Hoffman clamps, 27	5.40	$ 8.68

Approximate cost of 72 processing assemblies:

Fruit juice glasses, 6 dozen @ .40	$ 2.40	
Screw-cap capsule vials, 6 dozen	3.75	
Black molded caps, 6 dozen	1.00	
Corks, large, 6 dozen	7.56	
Corks, small, 6 dozen	1.69	
Glass rods, 27	.25	$16.65
Each assembly: $0.231		
Tripod	.55	
Small assembly tray, aluminum	.60	
Large assembly tray, cyprus	4.50	
Large assembly tray, aluminum	3.00	

Approximate cost of combined apparatus, $29.48 to $30.98.

CHEMICAL

BLOOD BROMIDE DETERMINATIONS: THEIR USE AND INTERPRETATION*

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THE critical level at which symptoms of bromide intoxication appear is the subject of considerable controversy. In addition to its diagnostic value the blood bromide determination has been used in estimating prognosis. This has led to conflicting statements, since many writers have attempted to correlate the symptoms of bromide intoxications with an absolute figure in terms of bromide content of blood and cerebrospinal fluid. Such disagreement is due to the use of chemical methods which do not selectively and accurately determine bromine, and to personal differences among patients which condition the absorption and excretion of bromides. It is the purpose of this paper to present data obtained by the most popular methods, to discuss sources of error and the biochemical and physiologic factors which are pertinent to the interpretation of the blood bromide concentration when used in diagnosis and treatment.

METHODS FOR DETERMINING BROMIDES IN BODY FLUIDS

Bromine is the most difficult halogen to determine, particularly in the presence of others. The exact chemical methods, whether volumetric or gravimetric, require elaborate equipment and laboratory facilities beyond those possessed by most hospitals and skill beyond that of the average laboratory technician. For the most part they require from six to twenty-four hours for completion; the disadvantage of this element is obvious when the results are needed in emergency situations.

Most clinical investigations of blood bromides have been made by adding gold chloride to a protein-free filtrate of serum. If bromide is found beyond that normally present (0.7 to 1.0 gamma per 100 c.c.), there is a color change in the mixture from yellow to brown (or even to brownish red) due to the formation of gold bromide, and the depth of color is proportionate to the concentration. This reaction is inversely analogous to that which occurs in vivo after the ingestion of bromides when the bromide ion is substituted in the body for the chloride ion. The gold chloride reaction was first used by Walter.[1] Walter's method was said to be specific for bromides, but was later shown to be affected by other halogens. Wuth[2] and Diethelm[3] further modified the procedure, recommending it for the diagnosis of bromide intoxication. Diethelm used color tubes containing sodium bromide treated with gold chloride and trichloracetic acid as standards and with them the comparison was made without optical assistance.

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Fremont-Smith, Dailey, and Sloan[4] compared Walter's method with that of Toxopeus[5] and found that there were large discrepancies in their results.

Wuth stated in his paper that there was considerable inherent error due to the adsorption of bromide by the serum proteins. This, he claimed, was of the order of 1 to 2 per cent in concentration of 25 mg. per 100 c.c., and from 11 to 14 per cent in a concentration of 200 mg. per 100 c.c. Katzenelbogen and Goldsmith,[6] who used modifications of the Walter test, experienced difficulty in matching the standard gold bromide with that in the serum. They attempted to obviate this by adding sodium chloride to the standard solution.

Without question the gold chloride method determines bromides, but, in addition, it may determine other substance as well, and thereby lose one of the chief values of a chemical examination, i.e., specificity.

Yates'[7] procedure is probably the simplest accurate method which does not require equipment beyond the scope of a good clinical laboratory. A protein-free preparation of the material being analyzed is placed in a nickel crucible with 25 per cent potassium hydroxide and evaporated to dryness. It is heated in an oven at 500° C. for twenty minutes, then cooled to 18° C. Sulfuric acid is added slowly, followed by a chromic-sulfuric acid mixture. Potassium iodide and starch solution are added, and the mixture is then aerated for five hours. At the end of that time, it is titrated with N/1,000 sodium thiosulfate. We have obtained consistent results and excellent recoveries with this method.

EXPERIMENTAL PROCEDURE

In the course of routine bromide determinations we have observed that the gold bromide reaction is subject to considerable error and, in spite of its simplicity, the potential error is enormous. Our experiments were devised to ascertain whether the gold chloride method would (1) yield duplicate results on the same material, (2) negative results in the absence of bromine, and (3) recover added bromides from serum. The effect of varying serum protein concentrations and of varying the bromide concentrations with respect to protein adsorption was also studied.

The procedure for blood bromide determinations followed in our experiments was that suggested by Diethelm, but three quantitations were made on each specimen of blood, employing for standard comparison (1) color tubes containing various dilutions of sodium bromide treated with gold chloride and graduated at intervals of 10 mg.; (2) a Bausch & Lomb visual type colorimeter, using standard solutions of gold bromide containing 1 or 2 mg. of sodium bromide per cubic centimeter according to the expected concentration of bromide in the unknown solution, and (3) a photoelectric comparator which had been standardized with ten solutions of sodium bromide treated with gold chloride. Using this apparatus, it was possible to refer all bromide concentrations to a single standard. The standard solutions were checked by the method of Yates for accuracy. The mean error in them was ±0.78 per cent. The bloods were filtered after being treated with trichloracetic acid to remove proteins. There were added to the filtrates, 0.5 per cent gold chloride in the proportion of 0.2 c.c. to each 1 c.c. of filtrate.

A series of bloods known to contain increased amounts of bromides were examined by the foregoing procedures. The findings were compared to determine whether it is possible to obtain duplicate values on the same specimen and to learn the error between several methods of comparing unknown concentrations of gold bromide with standard concentrations. The blood specimens were obtained from voluntary subjects of other experiments, who received sodium bromide by mouth and no other medication for periods ranging from ten days to six weeks. The ordinary diet with customary sodium chloride was eaten. Two determinations by each method were done, and each specimen was quantitated by the three methods described. The data from 20 specimens are presented in Table I, from which it can be seen that the visual comparison of gold chloride treated blood filtrates with color tubes is accompanied by considerable error. This is chiefly due to the impossibility of matching colors closely by this means, and in part to the fact that our color tubes represented intervals of 10 mg. of bromide. Using these tubes, closer intervals than 5 mg. could not be approximated by the eye. The range of values found by visual comparison under standardized illumination was from 20 to 155 mg. per 100 c.c., and the average difference between two determinations on the same serum was 19.8 per cent. The maximum difference was 37.5 per cent and the minimum was in one case 0.

When a Bausch & Lomb colorimeter was used for comparison, the bromide values could be estimated more closely. The range of concentration was from 21.4 to 156.4 mg. per 100 c.c. A number of specimens could not be matched exactly with the standard, appearing to be slightly off-color (Katzenelbogen and Goldsmith[6]). The average difference between two determinations was 6.1 per cent, the maximum 19.8 per cent and the minimum 0.4 per cent. Thus the use of the colorimeter reduced the error considerably, but the analyst is frequently confronted with bloods in which a comparison is very difficult or impossible even with this optical assistance. Except for the badly matching filtrates, the difference was 5.5 per cent or less, which limit is a considerable error for exact chemical determinations, but is much more acceptable than the values obtained by direct visual comparison.

The use of the photoelectric colorimeter further reduces the variation between two determinations on the same blood. The average difference in the twenty bloods reported here was 0.7 per cent. The maximum difference was 2.3 per cent and the minimum was 0. For clinical purposes this instrument offers relatively little advantage over the Bausch & Lomb colorimeter, but it entirely eliminates the individual factor in making comparisons; it allows reference of all specimens to a single standard and permits many determinations to be made in a very short time.

However, the close agreement between some of these determinations when made by colorimeter may be misleading, since in many laboratories the only criterion for control of a method is that it shall yield comparable results on the same material. The possibility of chlorides and other substances being determined along with bromides is not ruled out when such a criterion is used.

With respect to the use of prepared color tubes for comparison, as in Diethelm's procedure, it is probable that the error would be greater than that observed by us, since the intervals recommended are greater than 10 mg. at many points.

INFLUENCE OF SUBSTANCES OTHER THAN BROMIDES

A number of bloods were taken from patients who had been hospitalized for a long time and who had not received any bromide medication. These were subjected to the specific qualitative test of Belote,[8] and a gold chloride determination was made. As far as possible bromides were ruled out of the immediate past history of these individuals, either from the hospital record or from their own statement. Among the 76 bloods examined were several from reliable voluntary subjects who disclaimed bromide ingestion for six months or longer before the blood was drawn. Of this series no blood gave a positive qualitative eosin reaction, but 9 showed from 16 to 53 mg. of bromide by the gold chloride method. Two patients took no medication of any sort for four and six months, respectively, and the other 7 received various substances. Among the medication received by the patients who appeared to have increased blood bromides were the following substances: cysteine, diphenylhydantoin, liver extract, caffeine sodiobenzoate, wine of Bulgarian belladonna, thyroid, pituitary extract, and physiologic saline solution.

It is difficult to explain the positive findings in these cases unless the gold chloride reaction is nonspecific. It has been pointed out by Johnson and Partlow,[9] Tod,[10] and Katzenelbogen and Czarski[11] that chloride and iodides interfere with the reaction; this may explain the findings in several patients. If it is true that the gold chloride reaction is nonspecific, levels at which toxic symptoms appear must be disregarded, since some of the so-called blood "bromides" in many cases may actually be due to nonbromide material. Thus the use of the gold chloride reaction to follow the bromide treatment of epileptic patients with bromides may be fallacious.

To corroborate the positive findings in patients with blood bromides who had not been ingesting bromides, the medication which they had been receiving was added to bromine-free blood sera (1:20,000) and gold chloride tests were made. Diphenylhydantoin, pituitary extract, thyroid, potassium iodide, and Lugol's solution gave negative results by the eosin test, but produced slight degrees of color after gold chloride was added. The color produced was not adequate for comparison with a standard by a colorimeter, but the observation suggests that the gold chloride reaction is nonspecific for bromides.

ADSORPTION OF BROMIDES BY BLOOD PROTEINS

If we assume that the error which tends to lower the bromide values is chiefly due to protein adsorption, it would seem probable that the higher the concentration of proteins in the serum, the more adsorption would occur. Twenty bromine-free sera to which sodium bromide in concentrations varying from 25 mg. per 100 c.c. to 600 mg. per 100 c.c. had been added were examined. The total serum proteins were determined by the method of Greenberg;[12] the bromide concentration was determined by the Yates method and by the gold chloride method.

TABLE I

REPRODUCIBILITY OF RESULTS BY DIFFERENT METHODS OF COMPARISON WITH STANDARD

SPECIMEN NO.	DIRECT COMPARISON WITH COLOR TUBES		BY USE OF BAUSCH & LOMB COLORIMETER		BY USE OF PHOTOELECTRIC COLORIMETER	
	MG. PER 100 C.C.	PER CENT DIFFERENCE	MG. PER 100 C.C.	PER CENT DIFFERENCE	MG. PER 100 C.C.	PER CENT DIFFERENCE
A 3	70		74.3		74.3	
	95	26.3	76.2	2.4	74.7	0.5
A 7*	125		127.0		127.4	
	150	20.0	146.2	13.1	127.0	0.3
A 8	65		72.7		73.4	
	80	23.3	73.8	1.5	73.9	0.6
A 9	50		58.1		58.0	
	65	30.0	58.4	0.5	58.0	0
A 10*	70		68.6		68.9	
	85	17.6	59.8	12.6	68.6	0.4
A 11*	120		156.4		141.9	
	155	22.5	140.0	10.4	145.3	2.3
A 14	70		88.8		90.1	
	90	22.2	92.4	3.3	90.1	0
A 16	70		63.4		59.9	
	55	21.4	59.9	5.5	60.1	0.3
A 21	75		87.8		83.2	
	90	16.6	84.3	3.9	83.7	0.5
A 29*	90		125.3		106.9	
	110	18.1	107.0	14.6	106.9	0
A 30	115		136.3		136.3	
	135	14.4	130.0	4.6	139.2	2.0
A 31	115		119.0		117.4	
	140	17.8	116.3	1.3	118.0	0.5
B 13	55		55.4		55.2	
	65	15.4	57.8	4.1	55.4	0.4
B 14	70		67.2		66.2	
	70	0	66.1	1.6	66.6	0.6
B 15	80		98.4		95.9	
	110	37.5	98.6	5.3	96.3	0.4
B 16	20		21.4		22.4	
	30	33.3	26.7	19.8	22.9	2.0
B 41	45		47.4		47.6	
	55	18.1	48.3	1.0	48.1	1.0
B 43	80		87.6		89.3	
	95	15.7	88.0	0.4	90.1	0.8
B 44	100		103.5		102.4	
	105	4.7	105.7	1.1	103.1	0.6
B 45*	80		129.6		111.6	
	115	21.7	112.4	14.2	110.6	0.8
Average per cent		19.8		6.1		0.7

*These bloods could not be matched easily with standard.

It was found that the protein concentration has little relation to the amount of bromide recovered, since the values were almost evenly distributed with respect to the mean protein value and the mean bromide recovery value.

When recovery values were plotted against bromide concentration, it appeared that the higher the concentration the lower was the recovery of bromides from serum by the gold chloride method. Thus, it could seem that the error is greatest when exact information is most important due to the critical implications of high bromide values. No marked effect on the gold chloride method was observed by altering the acidity of either unknown or standard solutions within the limits which might be expected in the course of an ordinary determination.

DISCUSSION

The use of methods which give only an inaccurate estimate of the bromine concentration is unfortunate. The gold chloride method is not accurate, and its use for the initial diagnosis of intoxications may be adequate, but a simple, accurate method is needed for use in prognosis and in following treatment. It must be employed before an attempt to correlate the symptomatology with blood concentrations in bromide intoxication can be made.

This oversight may lead to more serious confusion than already exists. As a medicolegal test the value of the gold chloride method would be seriously questioned but the failure to consider individual variations in the ability to handle bromides is possibly more serious than the inaccuracies of the method. The danger of confusing bromide intoxications with other conditions has been pointed out frequently, but no one has expressed concern over confusing other stuporous conditions with bromide intoxication and administering large amounts of salt. We should consider the folly of giving 20 Gm. of salt daily to a person who has suffered head trauma and who has taken bromides to relieve the pain of a concussion or a subdural hemorrhage, or to one who is suffering from coma of renal origin. Elevated bromide contents in the blood are often found superimposed upon more serious conditions resulting from entirely different etiology.

In dealing with conditions in which bromides are represented, the determination of urine and blood chlorides, along with bromides, is a far better indication of the progress of treatment or of the validity of a diagnosis of bromide intoxication than isolated blood bromide determinations, but the clinical condition of the patient is more important than any laboratory finding. It is not our belief that blood bromide determinations should be abandoned, but rather that more accurate methods should be employed and that the limitations of laboratory findings should be recognized.

SUMMARY

Blood bromide concentrations are usually determined by a reaction in which gold chloride is added to a protein-free filtrate of serum, and the resulting colored substance is compared with quantitative standards. Most of the exact bromide methods are difficult and are beyond the scope of hospital and clinical laboratories. The gold chloride method has been examined critically, and sources of error are described. It is possible to obtain almost duplicate results

with some modifications of this method, but this is misleading, since it causes unwarranted faith to be placed in the results regardless of other sources of error. The use of a colorimeter can reduce the error considerably, but it has been our experience that it is appreciable. The reaction is probably not specific for bromides, since other chemically unrelated substances may cause a positive reaction. The concentration of blood bromides appears to be more important than the serum-protein concentration as a source of error. There have been reported anomalous findings of persons with extremely high blood bromides who showed no toxic symptoms, and of others who have demonstrated extreme bromide intoxication while the blood bromide was low, which may be related to the method used. The comparison of chloride and bromide levels in blood and urine is more valuable than isolated determinations of the blood bromide. Such data should be used as presumptive evidence. The clinical condition of the patient is a more valuable diagnostic and therapeutic index.

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STUDIES ON MELANURIA*

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MELANURIA usually occurs in the final metastasizing stage of malignant melanomas. In the urine a colorless or light-colored precursor of melanin, a water-soluble melanogen or chromogen, is excreted which in the presence of oxygen is readily transformed into the dark melanin precipitate. The usual laboratory tests to detect melanuria are based on this fact. An oxidizing agent is added to the urine, and the subsequent darkening is taken as a sign of melanuria. The recommended oxidizing agent is ferric chloride. As a second test bromine water is used. Most biochemical and clinical laboratory manuals and textbooks do not mention tests other than those for detection of melanin precursors. These tests, however, are neither sensitive nor specific, and they yield highly confusing and inconsistent results, as pointed out by Blackberg and Wanger in 1933.[1] Ferric chloride may form dark brown precipitates in normal urines if they are alkaline, and falsely positive results also can be obtained if drugs are excreted in the urine. Furthermore, these tests are hardly more sensitive reactions than the simple darkening of the urine exposed to air.

And yet, more sensitive and more specific qualitative chemical tests for the detection of melanogen have been known since 1887. In that year Thormählen[2] described the reaction which has been named after him, and which in our laboratory was found to be about 50 times as sensitive as the oxidation tests. So far the Thormählen test has never been found to be positive in the urine in any condition other than melanuria.

This test is performed in a manner similar to the Legal test for acetone, although it has no relation to it. A few drops of diluted solution of sodium nitroprusside are added to 3 to 5 c.c. of the urine, and then a few drops of a strong alkali solution. In our experience 10 per cent sodium hydroxide solution is the best alkalizing agent, better than ammonia or caustic soda. By alkalinization a deep ruby color results. This color is not characteristic for the melanogen. It is also formed by acetone and by creatinine. But if one acidifies with glacial acetic acid an azure blue color immediately appears which is specific for the melanogen. By addition of glacial acetic acid acetone turns deeper red; creatinine becomes yellow, then slowly green, and finally blue. Of course, Thormählen's test can be considered positive only if, through acidification, the color suddenly changes to blue.

Another even more sensitive, but less specific, test is Ehrlich's aldehyde reaction, which is known as the urobilinogen test. There is evidence that the

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positive Ehrlich reaction in melanuria is not due to the simultaneous presence of urobilinogen. The urobilinogen reaction disappears from the urine after its exposure to air for a short time (maximum after two hours), whereas in melanuria the test remains positive as long as does the Thormählen test, usually for several days. In fractionating the urine by different precipitation methods, the Ehrlich test is positive in those fractions which contain the melanogen, and negative in the others. Even preparations of melanogen which are purified chemically to a high degree are Ehrlich positive.[3]

The Ehrlich test was found in our laboratory to be about twice as sensitive as the Thormählen test. Therefore, melanogen may be detected with Ehrlich's reagent when the Thormählen reaction is still negative. However, in such a case the Ehrlich reaction as an indication of the presence of melanogen must be verified by concentrating the urine and then demonstrating a positive Thormählen test. In any case, the positivity of both reactions indicates the presence of melanogen with absolute certainty.

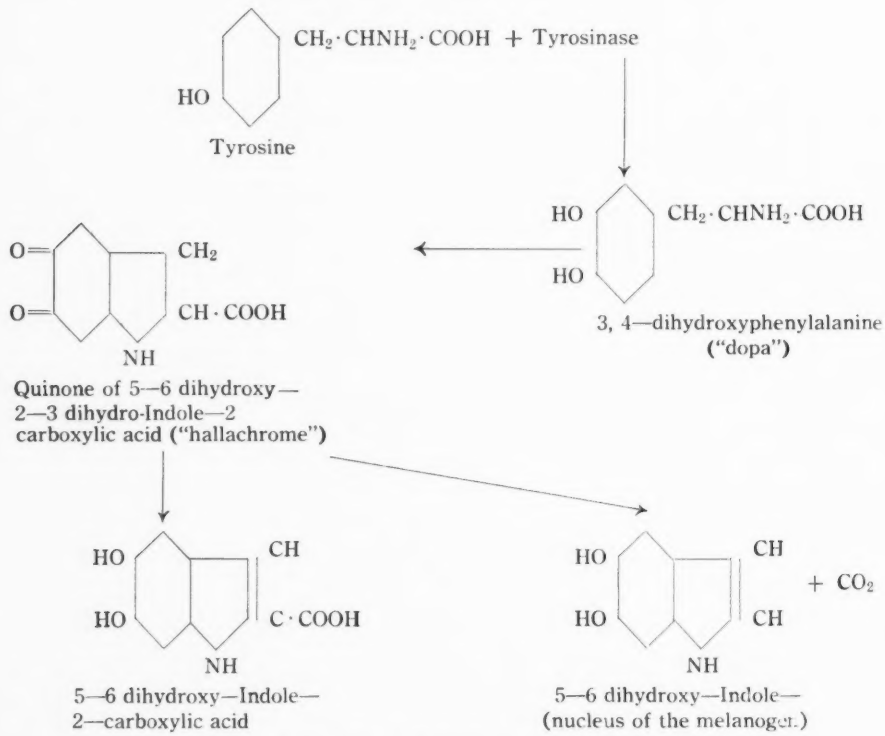


Fig. 1.—Formation of melanin precursors (Raper[4]).

It has been known for a long time that Thormählen's reaction is given only by indole and its derivatives. However, the origin of the indole compounds in the melanotic urine was not understood until Raper[4] in his fundamental work traced step by step the chemistry of melanin formation from tyrosine. Before that it was an accepted assumption that the source of the "Thormählen substance" is tryptophan as the only natural indole compound in the protoplasm.[5]

Raper presented evidence that indole derivatives are formed from tyrosine in the course of melanin formation, and it seems today that the only source of melanin pigment in the whole animal world is tyrosine.

In the course of oxidation of tyrosine to melanin by tyrosinase[4] or by ultraviolet light[6, 7] the first step is the formation of dihydroxyphenylalanine, or by abbreviation "dopa" (Fig. 1). The next step is the formation of a ring by the alanin side chain so that an indole nucleus results which is a condensed benzene and pyrrole ring. In 1935 Linnel and Raper[3] presented strong supporting evidence that the melanogen is the monopotassium salt of 5-6 dihydroxy-indole. In acknowledging the findings of Raper it became easy to understand that in addition to the indole derivatives, catechol compounds also (derivatives of dopa) were found as melanin precursors in melanotic urine.[8, 9]

EXPERIMENTAL

Most experiments were carried out with the urine of a 67-year-old patient from the service of Dr. George F. Dick. The patient presented the usual history of persons suffering from malignant melanomas. His left eye had been enucleated one year ago. He was admitted, very ill with jaundice and liver enlargement. His urine had been dark for several weeks. Only about three liters of this patient's urine could be collected since he was suffering from incontinence, and the daily urine obtained averaged 300 c.c. daily for ten days, after which time he died. Permission for autopsy was not obtained.

One urine specimen of another patient with a similar history was supplied by Dr. M. H. Ebert from the Rush Medical School, Chicago. This specimen amounted to only 70 c.c.

The material was first used for estimating the sensitivity of the different melanogen tests. This estimation was carried out by serial dilution of the urine with water and estimation of the threshold dilutions at which the reactions became negative. As mentioned in the introductory remarks, it was found that Thormählen's test was 50 times, and the Ehrlich test was 100 times, as sensitive as the ferric chloride test.

The material was used for answering two other clinically important questions: (1) Whether by simple methods the melanogen can be differentiated from indole and indican; and (2) whether the melanogen can be concentrated by fractionation of the urine so that smaller amounts may be detected in the fraction than is possible in the whole urine.

1. The qualitative chemical differentiation of the melanogen, indole, and indican was possible by applying the common laboratory tests for indole derivatives. As shown in Table I it was found that urine containing large amounts of indican does not give the Thormählen, Ehrlich, and Herter indole reactions, and vice versa. The melanogen does not react with Obermayer's and with Jolles' reagents. Indole itself displays the same color reactions as the melanogen, but with one remarkable exception: Herter's reaction most sensitive for indole is negative in the melanotic urine. This is an expedient to differentiate indole from the melanogen.

2. Concentration of melanotic urine for clinical purposes was carried out by Blackberg and Wanger,[1] but their method was based on the oxidation of

melanogen to melanin and not on the sensitive color reactions of indole. Fractionations for purposes of chemical identification of the melanogen were made by several authors.[3, 9, 10]

Testing all recommended methods of fractionation we found that the simplest and most effective method was precipitation with lead acetate.

TABLE I

QUALITATIVE COLOR TESTS OF INDOLE AND ITS DERIVATIVES OCCURRING IN THE URINE

TEST	INDOLE	INDICAN	MELANOGEN
Herter's naphthoquinone test	+	−	−
Ehrlich's aldehyde test	+	−	+
Thormählen's test	+	−	+
Nitroso-indole test	+	−	+
Obermayer's test	−	+	−
Jolles' test	−	+	−

The fresh urine was acidified with acetic acid to a pH of about 4.5, and lead acetate was added. The precipitate containing phosphates and sulfates was discarded. The clear filtrate, then, contained the total amount of melanogen. If this filtrate was alkalinized with ammonia to about pH 8, the newly formed lead precipitate took down most of the melanogen. After sharp centrifugation and separation from the supernatant fluid, the precipitate was ground up in small amounts of water. The suspension was decomposed by introduction of hydrogen sulfide. The precipitated lead sulfide was removed by filtration; the excess hydrogen sulfide by cautious heating. The clear yellow filtrate contained three to ten times as much melanogen as the original urine had in the same volume.

ADDITIONAL OBSERVATIONS

1. In the fractions obtained by precipitation with lead acetate the melanogen proved to be much more stable than in the original urine. Positive Thormählen and Ehrlich reactions could be demonstrated in these fractions after the samples were refrigerated in the dark for four months, covered with a cork stopper only, unprotected against oxidation. Obviously this stability of the melanogen was not due to the acidity of the fractions (pH 5) because an even better protection of the melanogen was obtained with barium hydroxide in slightly alkaline medium.[10] It seems rather that any kind of fractionation, consisting of rough chemical procedures, removes or inactivates a component of the urine which accelerates the oxidation of melanogen to melanin. It may be assumed that this factor is the enzyme of melanin formation which is present in pigmentary malignant tumors[11, 12] and which could be spilled over in the urine together with the melanogen.

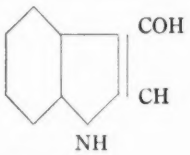
2. Dr. F. K. Oldham, of the Department of Pharmacology, using hypophysectomized frogs as test objects, demonstrated melanophore activity in our melanotic urine samples. Earlier experiments with similar results were carried out on animals which were not hypophysectomized.[13, 14] However, this assay is reliable on hypophysectomized frogs or fishes only.[15] Roughly concentrated fractions of the melanotic urine had no darkening effect on the hypophysectomized frog.

3. The originally light yellowish acid urine fractions with high melanogen content turned brilliant red on standing in the icebox. Similar observations were made as early as 1890,[16] and a contemporary thorough chemical study of the red substance in melanotic urine was made in 1936.[9]

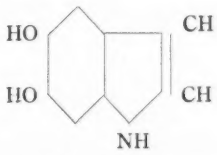
Chemical and spectrographic analysis of the red pigment in our laboratory showed that it is similar to the acid-soluble red pigment of human hair, first described by Arnow in 1938,[17] but is not identical with it. The detailed results of these experiments will be described elsewhere.

DISCUSSION

The fact that melanogen does not show indican reactions and that indican does not give positive indole reactions is in accord with the statement of Böhm.[18] In 1939 Böhm, in a different way, reached the conclusion that indigo blue formation, on which the indican tests are based, occurs only in those indole derivatives which have substituted C atoms on the pyrrole ring so that they can react there. The 5-6 dihydroxy-indole compound of Raper can react on the fifth and sixth C atom of the benzene ring but not on the pyrrole ring, and, therefore, does not yield indigo blue on oxidation (Fig. 2).



Indoxyl
Type of pyrrole—substituted indole derivatives
(positive indigo—blue reactions)



Nucleus of the melanogen (Raper)
Type of benzene—substituted indole derivatives
(positive indole reactions)

Fig. 2.

The unexpectedly negative Herter reaction of the melanogen which we obtained has not been previously reported.

By the concentration method described above, the test for melanuria has been rendered up to ten times as sensitive as is the Thormählen test and up to 500 times as sensitive as is the ferric chloride test in the original urine. It seems probable that the diagnosis of melanuria can be made considerably earlier in this way.

It has been claimed[1, 5] that the urine becomes dark only after the liver has been involved to a considerable degree in the metastatic process so that a functional insufficiency of the liver results. If this were true, if the melanuria indicated marked liver damage rather than growth of melanotic tumors anywhere in the body, there would be little hope of discovering the melanuria in an early state by concentration methods. However, there is strong evidence against this theory. By feeding indole derivatives which are not substituted on the pyrrole ring to normal animals and normal human beings, melanuria can be easily provoked, which is chemically identical with pathologic melanuria following malignant pigmentary growth.[18] Thus melanuria can be provoked in normal organisms without actual liver damage. It seems that melanuria depends only on the amount of melanogen present in the body. If this amount exceeds the threshold, either as a result of feeding or as a result of the metabolic process

in the tumor, melanogen is excreted through the kidneys. There is no reason to assume that exceeding of the threshold occurs suddenly with spilling over of large amounts of melanogen from the beginning. It is more probable that there is a gradual overstepping of the threshold similar to the case of glycosuria or indicanuria. In this case "latent melanuria" may precede macroscopically obvious melanuria by weeks or months. Therefore, in patients who are thought to have melanotic tumors, the concentration method as described above might be useful for an early diagnosis.

SUMMARY

1. The melanogen of melanotic urine can be differentiated from other indole compounds occurring in the urine by simple qualitative chemical tests.

2. By the use of Thormählen's and Ehrlich's reactions in a urine suitably concentrated, the test for melanuria can be rendered 500 times as sensitive as it is with the ferric chloride test in the original urine.

3. By rough chemical fractionation the melanogen becomes more stable than it was in the original urine.

4. The melanotic urine has melanophore activity which disappears after rough chemical treatment.

5. The red pigment formed in melanotic urine after acidification and the pigment of human red hair have similar properties.

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A RAPID METHOD FOR THE DETERMINATION OF SERUM PROTEIN*

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MANY indirect methods, designed to replace the time-consuming Kjeldahl procedure, have been proposed for the determination of serum protein. These have been reviewed by Peters and Van Slyke,[1] who conclude that the specific gravity method is the most reliable. This makes use of the linear relationship which exists between the specific gravity of the plasma or serum and the total protein content. The existence of such a relationship for human plasma was first demonstrated by Moore and Van Slyke.[2] Weech, Reeves, and Goettsch[3] then used dog's serum and found an even closer correlation. Kagan[4] examined specimens of human serum and derived an empirical relationship differing only slightly from those previously reported.

For determining the specific gravity, Moore and Van Slyke used a small pyknometer and an analytical balance. The falling-drop technique of Barbour and Hamilton,[5] developed previously, offers the advantage of being rapid as well as accurate; but the apparatus is relatively complex, and considerable experimental skill is required for its operation. Bing[6] described a method in which serum is diluted with measured amounts of 0.9 per cent sodium chloride until it acquires a specific gravity corresponding to 3.0 per cent protein, as indicated by an accurately adjusted float in the form of a glass bead. Instead of a single bead Simeone and Sarris[7] employed twenty-one glass beads made at random in large numbers and carefully selected according to their densities so as to form a graded series covering the range of serum protein values. In use, these beads were immersed in the serum, one at a time, until a bead was found with a specific gravity approximately equal to that of the serum.

The present paper describes a specific gravity method for the determination of serum protein which is both rapid and accurate. The essential apparatus consists of a single article of glassware of relatively small dimensions which can be calibrated to read directly in either specific gravity or protein content. Only ordinary glass-blowing skill is required for its construction.

METHOD

The apparatus consists of a specially constructed pipette (Fig. 1*b*), containing two hollow glass beads. One bead is slightly lighter and the other is slightly heavier than the range of sera to be examined. In use, the pipette is filled with serum and placed in an upright position, the beads coming to rest at points k and k'. It is then closed and inverted by rotating 180 degrees.

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After inversion the lighter bead will move upward while the heavier one descends, the ensuing collision occurring at a point which is determined primarily by the specific gravity of the liquid contained in the pipette. By observing the position of this point with reference to a properly calibrated scale engraved on the tube (Fig. 1*d*), the specific gravity of any sample falling within the range covered by the beads can be found.

In filling the pipette it is convenient to use a suction tube, into which a snugly fitting solid glass bead has been inserted. Using the bead as a valve in the usual way, the liquid can, with ease, be drawn up into the pipette and retained while a rubber band is stretched over the tip. The suction tube is then removed and the closure is completed after the manner of blood pipettes. The trap, *t*, is designed to catch air bubbles. The form shown in Fig. 1*a* does not require a rubber band, and air bubbles, if present, are removed through one of the stopcocks. In use, this type of pipette is not filled completely; a small amount of air is left in the capillary below the upper stopcock in order to permit expansion of the liquid. The capillary must have sufficient length to serve this purpose.

Rotation of the pipette is facilitated by use of a holder, arranged so that it may be turned through 180 degrees in a vertical plane. This consists of a light metal frame provided with a spring clip for holding the pipette, and is mounted on a horizontal axle, which in turn is supported on a base equipped with leveling screws. Movement of the frame is limited to 180 degrees by means of stops. For ordinary measurements, however, satisfactory results are obtained simply by inverting the pipette while it is held in the hands.

No particular size of pipette is required. Capacities of 1 or 2 c.c. have been found to be satisfactory. The bulb is made of standard wall tubing having an internal diameter of about 4 mm. and a length of 80 mm. in the case of the 1 c.c. pipette, and 5 mm. and 100 mm. for the larger size. The capillary stems have a bore of approximately 1 mm., with the exception of the portion immediately below the trap, which is blown out to about twice this size in order to permit freer passage of air bubbles into the trap. The diameter of the beads is somewhat less than one-half that of the tube in which they are enclosed. It is well to have them as nearly equal in size as possible. This is accomplished by preparing a number of beads and matching them according to size with the aid of a low-power microscope provided with a micrometer eyepiece.

The beads are blown from thin-walled, capillary, pyrex tubing. The desired density is obtained by collecting a small lump of glass on one end and grinding off a little at a time until the bead neither sinks nor rises rapidly when placed in a solution of potassium sulfate of predetermined specific gravity. It is then returned to the flame where the remaining portion of the lump is fused into the main body of the bead. When made in this way, a bead will, in general, have a thick wall at one end; but this is not detrimental to its usefulness. Final adjustment, if necessary, may be made by grinding the thick end. The ground area is fire-polished in a small flame at a relatively low temperature. This procedure not only gives a much higher yield of useful beads than mere random methods, but also results in a considerable saving in time.

The walls of the tube are indented at k and k' by heating the glass in spots 120 degrees apart, and pushing inward with a thin brass tool. These indentations provide seats in which the beads are brought to rest in the center of the tube. Considerable care is required in making them so as to reduce the area of contact to a minimum, and also to allow sufficient space for the liquid to pass freely around the bead at the beginning of an excursion.

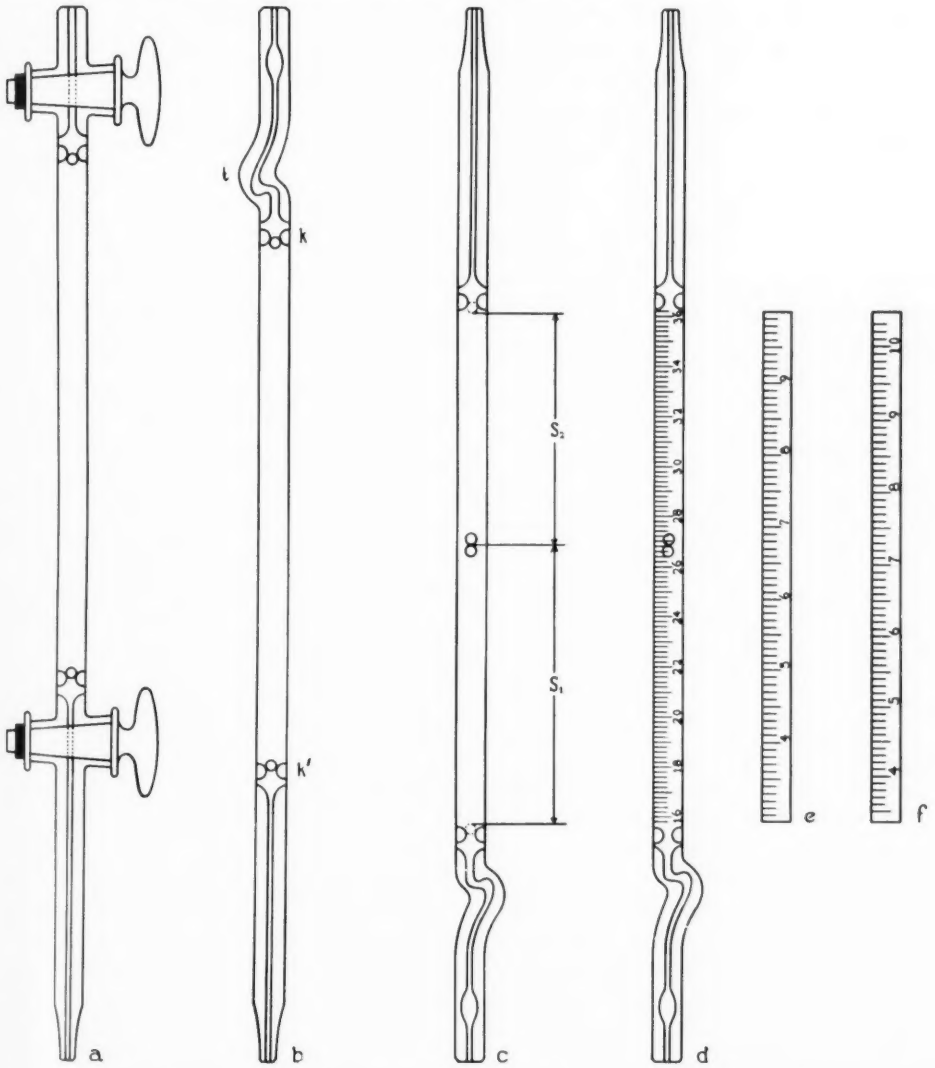

Fig. 1.—Specific gravity pipettes. *a*, With stopcocks; *b*, for rubber band closure; *c*, inverted, showing displacement of glass beads; *d*, graduated to read in specific gravity, figures representing second and third decimal places. Reading shown is 1.0268, corresponding to a serum protein content of 6.8 per cent. *e*, Scale for pipette (*d*) reading directly in percentage of serum protein; *f*, direct reading protein scale constructed from calibration curve in Fig. 2, $K' = 0.96$.

THEORY

When a small sphere falls under the action of gravity through a viscous fluid, the resistance increases with increasing rate of fall until it is equal and

opposite to the effect of gravity. Thenceforth, it has a constant velocity, for there is no resultant force acting upon it. This velocity is given by the well-known Stokes' law as

$$v = \frac{2g\ \alpha^2\ (d\text{-}D)}{9\eta} \tag{1}$$

where d is the density of the sphere, D the density of the fluid, η the coefficient of viscosity of the fluid, and α the radius of the sphere.

Stokes' law as given applies only to a spherical body falling through a fluid of infinite extension, but the velocity of a small sphere falling axially through a viscous liquid in a cylindrical tube may be expressed by the modified form

$$v = \frac{K\ (d\text{-}D)}{\eta} \tag{2}$$

K being a numerical constant for a particular tube. Then, replacing the velocity by its equivalent S/t, where S represents the distance traversed in the time, t, and applying the resulting formula to the motion of each bead in turn, two equations are obtained which may be combined to give the relation

$$\frac{S_1}{S_2} = K' \frac{D\text{-}d_1}{d_2\text{-}D} \tag{3}$$

where S_1 and S_2 are the distances traveled at constant velocity before collision by the light and heavy bead, respectively, and d_1 and d_2 are their densities. The value of K' is constant for a given apparatus, and is equal to unity when the two beads have the same effective radius. Since both beads are accelerated at the start in the same manner and reach their limiting velocities within a comparatively short distance, S_1 and S_2 may be taken to represent the entire distances traversed without introducing significant error.

An exact expression should take account of the repellent force exerted by one bead upon the other as the point of collision is approached. It can be shown that both are affected by this factor to the same extent only when their velocities are equal. Another factor is the "end effect," i.e., the effect of the ends of the tube on the velocity. The complete analysis, however, presents an involved problem in hydrodynamics.

Although formula (3) is obtained by an approximate treatment, it is sufficiently accurate to be used for purposes of calibration. It was tested in an extended series of experiments using matched beads of various sizes and densities in solutions of potassium sulfate as well as in serum, and found to hold, within rather narrow limits, over a wide range of fluid specific gravities. Typical results are shown in Fig. 2. It will be seen that the greatest deviations occur in the case of extreme values. None is of sufficient magnitude, however, to be of practical importance. It may be said that the accuracy with which the equation fits experimental values justifies the assumptions used in its derivation.

CALIBRATION

If the densities of the beads are known, a pipette may be calibrated by taking a single collision point reading on serum or a salt solution of known specific gravity. The reading should fall near the midpoint of the bulb, since

in this case errors of reading and inaccuracies in the formula will have a minimum effect. The beads are most conveniently calibrated, before being sealed in, by matching them with solutions of potassium sulfate and subsequently determining the specific gravities of the solutions with a pyknometer. With d_1 and d_2 thus found together with values for S_1 and S_2, the constant, K, in equation (3) is computed. Having evaluated K, it is possible to calculate the distances, S_1 and S_2, for any specific gravity lying within the range of the beads.

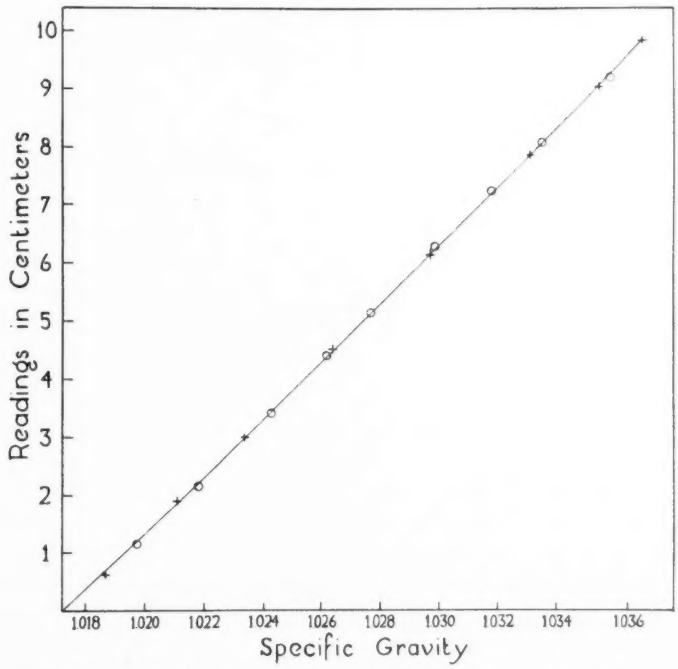

Fig. 2.—Typical calibration curve. Readings represent distance, S_1, traversed by light bead. Curve was drawn from points computed by equation (3) from bead densities and one collision point reading. Crosses indicate solutions of potassium sulfate; circles denote serum modified by dilution or evaporation.

A more direct method of calibration may be used in which collision point readings are taken on a series of standard solutions. Each point is marked with ink or a sharp wax pencil, and its distance, S_1, from the end of the bulb is measured by direct comparison with a millimeter scale. The specific gravities of the solutions are then plotted accurately on coordinate paper against the readings, S_1, and a smooth curve is drawn through the points. For ordinary work a curve based on four or five well-spaced points should suffice.

The two methods are compared in Fig. 2. It may be pointed out that the first method does not require the drawing of a curve. Regardless of which method is followed, a scale is readily constructed to read directly in specific gravity (Fig. 1*d*). Calibration in terms of percentage of protein is made with the aid of the empirical formula of Weech, and co-workers.[3] Integral protein percentages are substituted in the formula, and the corresponding specific gravities are calculated. The latter may be used in either of the foregoing methods for preparing a direct reading scale such as shown in Fig. 1*e*.

TEMPERATURE CORRECTION

In order to determine the influence of temperature, a pipette calibrated at 20° C. was filled with human serum and placed in a thermostatically controlled water bath so arranged that any predetermined temperature could be maintained within one-twentieth degree. Specific gravity readings were taken at intervals of five degrees, from 15° to 35°, with results as shown in Table I. Within the limits of experimental error the readings follow the thermal expansion of serum, indicating that the change in density of the serum is the only important factor involved. The expansion of the pyrex glass beads is about one-twenty-fifth that of serum and may be disregarded in dealing with a relatively narrow temperature range.

TABLE I

EFFECT OF TEMPERATURE ON PIPETTE READING

TEMPERATURE, °C.	SPECIFIC GRAVITY	PROTEIN (GRAMS PER 100 C.C.)
15	1.0310	8.3
20	1.0300	7.9
25	1.0288	7.5
30	1.0274	7.0
35	1.0258	6.4

It follows that a pipette which is calibrated at 20° C. and used at a temperature, t°, will read in specific gravity at t°/20° C. Between 20° and 30° the change in serum specific gravity averages about 0.00025 per degree centigrade. A reading taken within this temperature range may be reduced to specific gravity 20°/20° C. by adding 0.00025 to the observed specific gravity for each degree centigrade above 20. Such correction is accurate within one unit in the fourth decimal place. For clinical purposes an approximate correction may be applied by adding 0.1 to the percentage of protein for each degree rise in temperature.

RESULTS

In order to test the accuracy as well as the clinical practicability of the method, measurements were carried out on a large number of serum specimens, and the results were compared with those obtained by the pyknometer and falling-drop methods. A 2 c.c. pyknometer of the Nicol type provided with a graduated stem was used. This was found to be superior to the specific gravity bottle of Moore and Van Slyke[2] in accuracy, and ease of filling and cleaning. The falling-drop method followed the technique described by Barbour and Hamilton.[5] The blood samples were obtained by venepuncture from patients selected in most cases because they were known or believed to have abnormalities in their serum proteins. Typical results are summarized in Table II. Total protein estimations by the macro-Kjeldahl method are included for comparison.

Undoubtedly the pyknometer method is the most reliable one available for the determination of specific gravity. The average difference between the results obtained by the pyknometer and the specific gravity pipette is less than 0.0002. In the case of the falling-drop method the average difference is substantially the same, indicating that the method described here compares favorably in accuracy with the falling-drop technique.

The results obtained in the case of specimen No. 4 are of special interest. Repeated trials by the falling-drop method gave low values with a rather large variation among the individual determinations. This specimen was characterized by a cholesterol content of over 850 mg. per 100 c.c., 33 per cent of which was present in the form of the ester. Whether or not the failure to obtain satisfactory results can be ascribed to the unusually high content of these constituents was not ascertained. However, no difficulty was experienced with the specific gravity pipette.

TABLE II

COMPARISON OF RESULTS OBTAINED BY THE SPECIFIC GRAVITY PIPETTE WITH THOSE OBTAINED BY OTHER METHODS

SPECIMEN	SPECIFIC GRAVITY			PROTEIN (GRAMS PER 100 C.C.)	
	PYKNOMETER	FALLING DROP	PIPETTE	PIPETTE	KJELDAHL
1	1.0187	1.0188	1.0185	3.90	3.97
2	1.0203	1.0204	1.0204	4.57	4.61
3	1.0203	1.0203	1.0205	4.61	4.73
4	1.0207	1.0198	1.0207	4.68	4.96
5	1.0222	1.0222	1.0223	5.23	5.50
6	1.0234	1.0232	1.0233	5.58	5.68
7	1.0261	1.0260	1.0263	6.63	6.62
8	1.0286	1.0284	1.0285	7.40	7.43
9	1.0311	1.0312	1.0311	8.30	8.21
10	1.0333	1.0330	1.0336	9.18	9.01

DISCUSSION

Once the apparatus has been constructed and calibrated, the determination of serum specific gravity and total protein content becomes a matter of extreme simplicity. In making a determination it is necessary merely to fill the pipette, stretch a rubber band over the ends, invert it, and take the reading. In fact, a fair degree of accuracy can be obtained simply by inverting the pipette while it is attached to a rubber suction tube provided with a glass bead valve, thus dispensing with the use of a rubber band. For the greatest accuracy the use of the rotating holder is advised.

The pipette is cleaned and dried quickly by drawing water through it, followed by alcohol, ether, and air.

It is important to know the temperature at which a measurement is made. Ordinarily the reading of a thermometer placed near the apparatus is sufficient. In order to avoid warming the liquid, the pipette should be handled only by the capillary stems at points well removed from the bulb.

Certain other precautions should also be observed. Rotation must be carried out at such a rate that the beads will be kept in place by centrifugal force and yet not be thrown out of position as the inverted pipette is brought to rest. On account of the viscosity of serum this operation is not particularly critical and can be learned by a few trials. The most serious source of error arises from small air bubbles which adhere to the glass beads. These are readily seen in good light and when present must be shaken off. Difficulty from this source rarely occurs with serum used soon after centrifuging. It is most likely to occur with refrigerated serum, particularly if it is drawn into the pipette before coming to the temperature of the room.

Obviously, the method described here may be used to determine the specific gravity of any body fluid which is not opaque, if 1 c.c. or more is available. Various ranges may be covered by preparing glass beads of the required specific gravity. Furthermore, preliminary work has shown that the albumin and globulin fractions of serum may be determined separately by a modification of this method.

SUMMARY

1. A new specific gravity method for the determination of serum protein has been developed.

2. The method is rapid and accurate, and has the advantage of being direct reading.

3. The essential apparatus consists of a single article of glassware, the construction, calibration, and use of which are described in detail.

I am indebted to Dr. Newton Evans, of the Los Angeles County General Hospital, for his assistance in obtaining many serum specimens used in this work.

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DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TISSUE: Modification of Mallory's Phosphotungstic Acid-Hematoxylin Stain for Formaldehyde-Fixed Tissues, Peers, J. H. Arch. Path. 32: 446, 1941.

1. Deparaffinize sections and bring down to water.
2. Mordant three hours in a saturated aqueous solution of mercuric chloride in a paraffin oven at 57° C. Rinse briefly. The mercuric chloride solution may be used repeatedly.
3. Place in compound solution of iodine for five minutes. Rinse.
4. Place in 5 per cent aqueous solution of sodium thiosulfate for five minutes. Rinse.
5. Place in 0.25 per cent potassium permanganate for five minutes. Rinse.
6. Place in 5 per cent aqueous oxalic acid for five minutes. Wash well.
7. Stain overnight in phosphotungstic acid hematoxylin.
8. Wash briefly in tap water, dehydrate in alcohol or acetone, clear in xylol, and mount in balsam.

This technique gives results almost identical with those of the original method as applied to tissue fixed in Zenker's solution. Neuroglia, fibroglia, and myoglia fibrils, blepharoplasts, nuclei, red blood cells, and fibrin are deep blue, while collagen is brownish red.

TULAREMIA, Lawless, T. K. Arch. Dermat. & Syph. 44: 147, 1941.

The following antigen is proposed:

The pus from an unopened gland is aspirated and divided into three equal portions. These separate portions are triturated with ten volumes of acetone, alcohol, and 0.4 per cent saline solutions. The mixture is allowed to digest for twenty-four hours, with frequent agitation. The supernatant fluids are pipetted off and mixed. The alcohol and acetone are allowed to evaporate, and the original volume is made up with 0.4 per cent saline solution. The solution is sterilized on a water bath at 60° C. for two hours and twenty-four hours later for one hour. Tests for sterility are performed on agar plates. Phenol, 0.25 per cent, is then added.

As a test material, 0.1 c.c. is introduced intradermally, and the reaction is read at the end of one-half hour. A large erythematous, edematous wheal with pseudopodia in some cases will result in a case of the disease. This reaction remains for twenty-four or more hours, depending on the severity of the condition. A control subject should show either no reaction or one that disappears in ten to fifteen minutes. In patients presenting nonspecific reactions, the wheal disappears in from one to two hours. The earliest time at which a positive result was obtained was one day after onset of symptoms. Two weeks later a positive reaction was obtained with a tularemia agglutination test with a 1:1,280 dilution. The oldest condition was recognized five months after infection and had been diagnosed and treated as influenza, pneumonia, and empyema.

This antigen also agglutinates the red blood cells of infected persons and produces a specific histiocytic response in the local tissues.

PLASMA PROTEINS, Simple Method for Estimating, Walther, W. W. Lancet 241: 337, 1941.

Materials: Pyrex boiling tubes, 170 mm. by 28 mm., marked by a ring at 25 cm.

"Antibump" device consisting of 5 mm. of glass tubing fused to the end of a glass rod 160 mm. long.

Pure, nitrogen-free sulfuric acid.

Pure, nitrogen-free potassium persulfate saturated solution.

Nessler's solution (special for blood testing).

Nitrogen standard. Pure ammonium sulfate, 0.03776 Gm. per liter of distilled water containing 7 c.c. of pure sulfuric acid. It is convenient to prepare this by diluting 8 c.c of a 0.472 per cent solution of the salt to 1 liter.

Analyses may be done on heparinized plasma (using the minimum of heparin) or on serum.

Method: After washing out the boiling tube with distilled water, about 0.5 c.c. of water collects in it. Using the hemoglobin pipette, wash out 20 c.c. of serum or plasma into this water. Add 0.25 c.c. of pure sulfuric acid and mix. Boil gently over by-pass flame of Bunsen burner with "antibump" rod in position. When thick white fumes rise and fill the tube, cover with a watch glass and boil for three minutes. The solution is then quite black. Remove the flame for two minutes, and then add 0.5 c.c. of saturated potassium persulfate solution. Mix with antibump rod and replace flame. Boil without watch glass until thick white fumes rise again, then replace the watch glass and boil for two minutes longer, after which the solution will be clear and colorless. Cool, dilute with distilled water to the 25 c.c. mark, holding the tube with the 25 c.c. mark at eye level and remove the antibump rod. Replace the rod and wash well in the solution. Use any convenient volume (the author uses 5 c.c. of the solution placed in an ordinary test tube), and add 2 c.c. of Nessler's solution. A similar volume of standard solution is treated in the same way. Mix by inverting twice and compare at once in the colorimeter. Equal color indicates 1,000 mg. of total nitrogen per 100 c.c. of sample. A direct reading may be obtained by fixing the unknown at 10 and moving the standard to match. The reading of standard times 100 equals milligrams of total nitrogen per 100 c.c. of sample. The reading is greatly facilitated by placing a spectrum blue color filter over the eyepiece. Unless gross nitrogen retention is suspected, a subtraction of 50 mg. to cover the nonprotein nitrogen gives the figure for protein nitrogen which, when multiplied by 6.25/1,000 gives the protein percentage. The reagents should be tested occasionally by blank estimations.

If a colorimeter is not available, a comparator or similar instrument can be used, but the reading of the yellow Nessler solution is greatly helped by using a blue light filter. The accuracy of the method depends on individual skill in color matching, but it is well within the limits necessary for clinical work.

DIPHTHERIA, Present Status of the Immediate Tellurite Test for, Bierman, H. R., and Maxwell, R. W. J. A. M. A. **117**: 1255, 1941.

The potassium tellurite procedure for the differential diagnosis of diphtheria, as suggested by Manzullo, was applied to 117 patients exhibiting membranes of the throat.

The test was frequently positive on nondiphtheritic membranes but was falsely negative only twice.

A negative tellurite reaction, if it contradicts the clinical impression, is not sufficiently reliable to justify one in withholding antitoxin therapy.

It is felt that the technical difficulties of applying the test at present, and the frequency with which both false positive and false negative reactions are obtained, make it unsatisfactory as a routine guide for the therapy of diphtheria. Refinements in technique might admittedly enhance its usefulness.

SYNOVIAL FLUID and Synovial Membrane Abnormalities Resulting From Varying Grades of Systemic Infection and Edema, Coggeshall, H. C., Bennett, G. A., Warren, C. F., and Bauer, W. Am. J. Med. Sc. **202**: 486, 1941.

From a study of 156 post-mortem synovial fluids and 49 synovial tissue specimens obtained from persons dying with varying degrees of infection and edema, the following conclusions can be drawn:

1. The amount of synovial fluid in the knee joints of persons with peripheral edema is usually increased.

2. The synovial fluid obtained from the joints of edematous patients contains fewer nucleated cells than does normal fluid and its total solids, viscosity, protein, and mucin contents are reduced.

3. Total nucleated cell counts and the absolute number of neutrophiles in synovial fluid specimens obtained from patients dying with varying degrees of infection are frequently increased. The most marked cytologic abnormalities are observed in patients dying with severe infections and associated septicemias.

4. An increase in the absolute number of neutrophiles is a more reliable indication of the existence of synovial tissue inflammation than are increased total nucleated cell counts or random biopsies.

5. The synovial fluid abnormalities resulting from synovial tissue infections vary with the severity of the synovial lesion and its proximity to the joint space.

6. The results of the present study suggest that the joint aches and pains occurring in various infectious diseases may be the result of synovial tissue inflammatory changes caused by blood-borne infections. The majority of such inflammatory lesions are presumably mild in nature and, therefore, resolve readily. The marked synovial tissue reactions observed in patients dying with septicemia are of the type observed in early cases of specific infectious or septic arthritis.

TRANSFUSIONS, Infusions of Blood and Other Fluids Via the Bone Marrow, Tocantins, L. M., O'Neill, J. F., and Jones, H. W. J. A. M. A. 117: 1229, 1941.

Citrated blood and physiologic solution of sodium chloride have been infused through the marrow of a tibia or a femur of 9 infants in whose treatment intravenous infusions were urgent but impossible. In 2 with congenital anemia and erythroblastosis, it was not possible to infuse any blood by this method. No local or constitutional reactions, immediate or delayed, have been disclosed by clinical and roentgen-ray examinations after these infusions. When clearly indicated, the intramedullary route for parenteral therapy is feasible and useful.

BLOOD IODINE: Naturally Occurring Iodine Fractions and Their Chemical Behavior, Bassett, A. M., Coons, A. H., and Salter, W. T. Am. J. Med. Sc. 202: 516, 1941.

The protein-bound iodine in the blood plasma of man and of animals was found to reside chiefly in the traditional albumin fraction. This iodine was subject to fluctuations dependent upon thyroid activity. Such fluctuations, in absolute terms, were due chiefly to a thyroxine-like moiety thereof. The moiety resembling diiodotyrosine might increase proportionately, but it contributed relatively little to the absolute change from the normal level. Accordingly, total protein-bound iodine fluctuated largely with thyroxine-like iodine. In myxedema the thyroxine-like fraction practically disappeared.

Despite variations in this protein-bound iodine, the ionized iodine (inorganic) appeared to be rather low and approximately constant under the conditions of this study, regardless of the state of thyroid activity. Nevertheless, it was found to increase markedly when extraordinary, though perhaps very small, amounts of iodine entered the organism. Simultaneously a false increase in the protein-bound iodine occurred which could be reproduced by the addition of iodide to plasma in vitro.

The results indicate that the protein-bound thyroxine-like moiety of the plasma iodine is a good objective index of circulating thyroid hormone. They suggest, also, that plasma protein-bound iodine might be used clinically to confirm physiologic thyroid status.

BLOOD IODINE: Its Relation to Thyroid Function in 100 Clinical Cases, Salter, W. T., Bassett, A. M., and Sappington, T. S.

A series of 100 cases of suspected thyroid disturbance and 10 control patients have been analyzed from the standpoint of final clinical diagnosis, basal metabolic rate, and plasma protein-bound iodine. When the first two of these criteria are in agreement, there is a close correlation between the plasma iodine and the basal metabolic rate. Such cases amount to 71 per cent of the entire group studied. Of the remaining 20 per cent, the basal metabolic rate often did not clearly reflect the clinical status and the plasma protein-bound

iodine proved much more reliable. This was particularly true in the group of "Graves' without hyperthyroidism" in which the basal metabolic rate ranged from −20 to 40 per cent, but the plasma protein-bound iodine was normal. These data, therefore, constitute additional evidence for the possible dissociation of physiologic hyperthyroidism and clinical Graves' disease. In hypothyroidism plasma protein-bound iodine appears to be a highly reliable criterion for confirming lack of thyroid hormone, even when full-blown myxedema is absent.

It has been emphasized that the simplified chemical procedures employed are partly empirical. Their use has been justified by their consistency with clinical data, but due care should be exercised in comparing the data with the results obtained by other methods.

BLOOD: The Use of Rabbit Thrombin as a Local Hemostatic, Lozner, E. L., MacDonald, H., Finland, M., and Taylor, F. H. L. Am. J. Med. Sc. 202: 593, 1941.

Observations are reported indicating that rabbit thrombin is of distinct value in the control of hemorrhage from small wounds induced by a standard trauma in normal persons and in 9 patients with and in 2 patients without hemorrhagic diatheses bleeding spontaneously from small wounds.

The material apparently accomplishes its results by the instantaneous precipitation of fibrin.

No toxic manifestations were observed following the application of rabbit thrombin to the small wounds.

It would appear desirable that these observations concerning the use of rabbit thrombin as a local hemostatic be extended.

BLOOD: The Thrombic Activity of a Globulin Fraction Derived From Rabbit Plasma, Taylor, F. H. L., Lozner, E. L., and Adams, M. A. Am. J. Med. Sc. 202: 585, 1941.

The observation of Parfentjev that rabbit plasma contains a substance or physiologic activity capable of converting fibrinogen to fibrin is confirmed.

Parfentjev's material is apparently a pseudoglobulin which possesses true thrombic activity.

It may be differentiated from thromboplastin by its manner of preparation and its independence of calcium-ion concentration in its action on fibrinogen.

Methods of preparing the material in a stable powdered form are presented.

BLOOD CLOTTING: A Globulin Fraction in Rabbit's Plasma Possessing a Strong Clotting Property, Parfentjev, I. A. Am. J. Med. Sc. 202: 578, 1941.

A description is given of a globulin fraction, isolated from rabbit plasma, which possesses the property of clotting plasma.

It has been found that the "toxicity" of this fraction on intravenous injection is due to intravascular clotting.

Clotting globulin could be separated from that fraction of rabbit plasma which contains the major portion of the immune bodies.

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